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Transmitted herewith for filing is the patent application of Patricia A. BILLING-MEDEL, Maurice COHEN, Tracey L. COLPITTS, Paula N. FRIEDMAN, Mark HAYDEN, Michael R. KLASS, Lisa ROBERTS-RAPP, John C. RUSSELL and Stephen D. STROUPE for REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE GASTROINTESTINAL TRACT, which is a continuation-in-part of U.S. application serial no. 08/829,754, filed March 31, 1997.

Enclosed are:

- 9 sheet(s) of X formal informal drawing(s).
- A claim for foreign priority under 35 U.S.C. § 119/363 in
 a separate document the declaration.
- A claim for priority under 35 U.S.C. § 119(e)(1) in
 a separate document the declaration.
- A certified copy of the priority document.
- Verified Statement(s) Claiming Small Entity Status.
- Other:

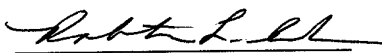
The declaration of the inventor X is enclosed X unsigned.

The fee has been calculated as follows:

A. Basic Application Fee		\$790.00
B. Total Claims 18 - 20 = 0	x \$22.00	0.00
C. Independent Claims 12 - 3 = 9	x \$82.00	738.00
D. If multiple dependent claims present, add	\$270.00	0.00
E. Total Application Fee (Total of A, B, C, & D)	=	1528.00
F. If verified statement of small entity status is enclosed, reduce Total Application Fee by 50%		NA
G. Application Fee Due (E - F)	=	1528.00
H. Assignment Recording Fee of \$40.00 if assignment document is enclosed	\$40.00	NA
I. TOTAL FEE (G + H)		\$1528.00

Respectfully submitted,

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REAGENTS AND METHODS USEFUL FOR DETECTING
DISEASES OF THE GASTROINTESTINAL TRACT

Cross-Reference to Related Application

5 This application is a continuation-in-part of U.S. application Serial No. 08/829,754, filed March 31, 1997, from which priority is claimed pursuant to 35 U.S.C. §120 and which is incorporated herein by reference in its entirety.

Background of the Invention

10 The invention relates generally to detecting diseases of the gastrointestinal tract organs, and more particularly, relates to reagents such as polynucleotide sequences and the polypeptide sequences encoded thereby, as well as methods which utilize these sequences, which are useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining predisposition to diseases and
15 conditions of the GI tract such as cancer.

 The organs of the GI tract include the esophagus, stomach, small and large intestines, rectum and pancreas. Of the approximately 225,900 new cases of GI tract cancer projected for the United States during 1996, 131,200 will be due to colorectal cancer. Further, GI tract cancers will account for approximately 127,070
20 related deaths (American Cancer Society statistics). In addition to its high incidence, GI tract cancers can be extremely lethal; for example, greater than 97% of pancreatic cancer patients will die of the disease. H.J. Wanebo, et al., Cancer 78:580-91 (1996).

 Generally, the early detection of GI tract cancers at a pre-invasive stage
25 dramatically reduces disease-related mortality. However, only few GI tract cancers are detected at this stage. For example, only 37% of colorectal cancers are detected at this stage by screening for premalignant polyps which can be removed before they progress to cancer. The primary methods used for colorectal cancer screening are fecal occult blood testing (FOBT) and flexible sigmoidoscopy. A. M. Cohen et
30 al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, pp. 929-977, Philadelphia, PA: J/B. Lippincott Co. (1993). Although FOBT is noninvasive, simple and inexpensive, its sensitivity is low; for example, sensitivity for detecting colorectal cancer was only 26% in one study. D.A. Ahlquist et al., JAMA 269: 1262-1267 (1993). Further, although flexible sigmoidoscopy is highly sensitive
35 for detecting early cancer and precursor polyps, it is invasive, costly, and too technically demanding to be used for routine screening. D.F. Ransohoff, et al.,

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JAMA 269: 1278-1281 (1993). In addition, only eight percent (8%) of pancreatic cancers and eighteen percent (18%) of stomach cancers are detected at a pre-invasive stage (American Cancer Society statistics). Thus, the need exists for improved screening methods for detection of GI tract diseases such as cancer.

5 The standard procedures currently used for establishing a definitive diagnosis for a GI tract cancer include barium studies, endoscopy, biopsy and computed tomography (CT). These procedures are invasive and costly. Moreover, an erroneous diagnosis can result from any of these procedures due to technical reasons, the subjective interpretation of results, or lack of sensitivity of the
10 procedure. M. F. Brennan, et al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, pp. 849-882, Philadelphia, PA: J.B. Lippincott Co. (1993).

 After the diagnosis of a particular GI tract cancer is confirmed, staging is performed to determine the anatomic extent of the disease. Staging is performed by a pathologist on tissue obtained by biopsy and/or surgery. Accurate staging is
15 critical for predicting patient outcome and providing criteria for designing optimal therapy. Inaccurate staging can result in poor therapeutic decisions and is a major clinical problem in colorectal cancer. A need therefore exists for more sensitive diagnostic procedures for staging GI tract cancers.

 While surgical resection of the affected organ is typical therapy for a
20 majority of patients diagnosed with GI tract cancers, some patients undergo radiation and/or chemotherapy. All of these patients need to be monitored in order to evaluate their response to therapy and to detect persistent or recurrent disease and distant metastasis. A variety of markers including CEA and CA 19-9 can be assayed and the assay results used to monitor a patient's progress in conjunction
25 with radiological procedures and colonoscopy. E.L. Jacobs, Curr. Probl. Cancer 15 (6):299-350 (1991). These monitoring techniques, however, have failed to provide an accurate and effective means to monitor the progress of these patients.

 Assays based upon the appearance of various disease markers in test samples such as blood, plasma or serum obtained by minimally invasive techniques, could
30 provide low-cost and accurate information to aid the physician in diagnosing disease such as cancer, in selecting a therapy protocol, and in monitoring the success of the chosen therapy. Such markers have been placed into several categories. The first category contains those markers which are elevated in disease. Examples include human chorionic gonadotropin (hCG) which is elevated in testicular cancer and
35 trophoblastic disease, and alpha fetoprotein (AFP) which is elevated in hepato-cellular carcinoma (HCC). E.L. Jacobs, supra. The second category includes qualitatively

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There are yet other examples of detecting disease markers in an inappropriate bodily compartment. In the case of metastatic cancer, the blood, bone marrow or lymph nodes may contain cells which have originated from the primary tumor and which may express mRNA or protein markers representative of the primary tumor. For example, CEA and PSA have been demonstrated immunohistochemically in lymph nodes or bone marrow of patients with metastatic colorectal cancer and prostate cancer, respectively. B.R. Davidson, et al., Cancer 65:967-970 (1990); J.L. Mansi, et al., J. Urol., 139:545-548 (1988). In addition, RT-PCR has detected CEA and PSA mRNAs at distant sites in patients with colon and prostate cancer, suggesting the presence of metastatic cells. M. Gerhard, et al., J. Clin. Oncol. 12:725-729 (1994); A.E. Katz, et al., Urology 43:765-775 (1994). Other compartments in which the inappropriate appearance of normal gene products may be indicative of disease include but are not limited to, whole blood, urine, saliva, and stool. Currently, no universally acceptable marker(s) exist(s) for the early detection of pancreatic, stomach, and esophageal cancers. Further, improved markers are needed to detect colorectal cancer.

It therefore would be advantageous to provide specific methods and reagents for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or 35 determining predisposition to diseases and conditions associated with the GI tract or to indicate possible predisposition to these conditions. Such methods would include

assaying a test sample for products of a gene which are overexpressed in GI tract diseases and conditions such as cancer. Such methods may also include assaying a test sample for products of a gene alteration associated with the GI tract disease or condition. Such methods may further include assaying a test sample for products of a gene whose distribution among the various tissues and compartments of the body have been altered by a GI tract-associated disease or condition such as cancer. Useful reagents include polynucleotide(s), or fragment(s) thereof which may be used in diagnostic methods such as reverse transcriptase-polymerase chain reaction (RT-PCR), PCR, or hybridization assays of mRNA extracted from biopsied tissue, blood or other test samples; polypeptides or proteins which are the translation products of such mRNAs; or antibodies directed against these proteins. Drug treatment or gene therapy for diseases or conditions of the GI tract then can be based on these identified gene sequences or their expressed proteins, and efficacy of any particular therapy can be monitored. Furthermore, it would be advantageous to have available alternative, non-surgical diagnostic methods capable of detecting early stage GI tract disease such as cancer.

Summary of the Invention

The present invention provides a method of detecting a target CS194 polynucleotide in a test sample which comprises contacting the test sample with at least one CS194-specific polynucleotide and detecting the presence of the target CS194 polynucleotide in the test sample. The CS194-specific polynucleotide has at least 50% identity with a polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, SEQUENCE ID NO 6, SEQUENCE ID NO 7, SEQUENCE ID NO 8, SEQUENCE ID NO 9, SEQUENCE ID NO 10, SEQUENCE ID NO 11, SEQUENCE ID NO 12, SEQUENCE ID NO 13, SEQUENCE ID NO 14, SEQUENCE ID NO 15, SEQUENCE ID NO 16, SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20 ("SEQUENCE ID NOS 1-20"), and fragments or complements thereof. Also, the CS194-specific polynucleotide may be attached to a solid phase prior to performing the method.

The present invention also provides a method for detecting CS194 mRNA in a test sample, which comprises performing reverse transcription (RT) with at least one primer in order to produce cDNA, amplifying the cDNA so obtained using CS194 oligonucleotides as sense and antisense primers to obtain CS194 amplicon, and

detecting the presence of the CS194 amplicon as an indication of the presence of CS194 mRNA in the test sample, wherein the CS194 oligonucleotides have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof. Amplification can be performed by the
5 polymerase chain reaction. Also, the test sample can be reacted with a solid phase prior to performing the method, prior to amplification or prior to detection. This reaction can be a direct or an indirect reaction. Further, the detection step can comprise utilizing a detectable label capable of generating a measurable signal. The detectable label can be attached to a solid phase.

10 The present invention further provides a method of detecting a target CS194 polynucleotide in a test sample suspected of containing target CS194 polynucleotides, which comprises (a) contacting the test sample with at least one CS194 oligonucleotide as a sense primer and at least one CS194 oligonucleotide as an anti-sense primer, and amplifying same to obtain a first stage reaction product; (b) contacting the first stage
15 reaction product with at least one other CS194 oligonucleotide to obtain a second stage reaction product, with the proviso that the other CS194 oligonucleotide is located 3' to the CS194 oligonucleotides utilized in step (a) and is complementary to the first stage reaction product; and (c) detecting the second stage reaction product as an indication of the presence of a target CS194 polynucleotide in the test sample. The CS194
20 oligonucleotides selected as reagents in the method have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof. Amplification may be performed by the polymerase chain reaction. The test sample can be reacted either directly or indirectly with a solid phase prior to performing the method, or prior to amplification, or prior to detection.
25 The detection step also comprises utilizing a detectable label capable of generating a measurable signal; further, the detectable label can be attached to a solid phase. Test kits useful for detecting target CS194 polynucleotides in a test sample are also provided which comprise a container containing at least one CS194-specific polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or
30 complements thereof. These test kits further comprise containers with tools useful for collecting test samples (such as, for example, blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, swabs, cups, and the
35 like, may optionally be treated to avoid denaturation or irreversible adsorption of the

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sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens.

The present invention also provides a purified polynucleotide or fragment thereof derived from a CS194 gene. The purified polynucleotide is capable of
5 selectively hybridizing to the nucleic acid of the CS194 gene, or a complement thereof. The polynucleotide has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof. Further, the purified polynucleotide can be produced by recombinant and/or synthetic techniques. The purified recombinant polynucleotide can be contained within a
10 recombinant vector. The invention further comprises a host cell transfected with the recombinant vector.

The present invention further provides a recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from CS194. The nucleic acid sequence has at least 50% identity with a sequence selected
15 from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof. The nucleic acid sequence is operably linked to a control sequence compatible with a desired host. Also provided is a cell transfected with this recombinant expression system.

The present invention also provides a polypeptide encoded by CS194. The
20 polypeptide can be produced by recombinant technology, provided in purified form, or produced by synthetic techniques. The polypeptide comprises an amino acid sequence which has at least 60% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO 41, SEQUENCE ID NO 42, SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, SEQUENCE ID NO 46,
25 SEQUENCE ID NO 47, SEQUENCE ID NO 48, SEQUENCE ID NO 49 (“SEQUENCE ID NOS 41-49”), and fragments thereof.

Also provided is an antibody which specifically binds to at least one CS194 epitope. The antibody can be a polyclonal or monoclonal antibody. The epitope is derived from an amino acid sequence selected from the group consisting of
30 SEQUENCE ID NOS 41-49, and fragments thereof. Assay kits for determining the presence of CS194 antigen or anti-CS194 antibody in a test sample are also included. In one embodiment, the assay kits comprise a container containing at least one CS194 polypeptide having at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. Further, the
35 test kit can comprise a container with tools useful for collecting test samples (such as blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth

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for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials such as papers, cloths, swabs, cups, and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also
5 may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Also, the polypeptide can be attached to a solid phase.

Another assay kit for determining the presence of CS194 antigen or anti-CS194 antibody in a test sample comprises a container containing an antibody which
10 specifically binds to a CS194 antigen, wherein the CS194 antigen comprises at least one CS194-encoded epitope. The CS194 antigen has at least about 60% sequence similarity to a sequence of a CS194-encoded antigen selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. These test kits can further
15 comprise containers with tools useful for collecting test samples (such as blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also may be treated with, or contain,
20 preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. The antibody can be attached to a solid phase.

A method for producing a polypeptide which contains at least one epitope of CS194 is provided, which method comprises incubating host cells transfected with an expression vector. This vector comprises a polynucleotide sequence encoding a
25 polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 60% identity with a CS194 amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

A method for detecting CS194 antigen in a test sample suspected of containing CS194 antigen also is provided. The method comprises contacting the test sample with
30 an antibody or fragment thereof which specifically binds to at least one epitope of CS194 antigen, for a time and under conditions sufficient for the formation of antibody/antigen complexes; and detecting the presence of such complexes containing the antibody as an indication of the presence of CS194 antigen in the test sample. The antibody can be attached to a solid phase and may be either a monoclonal or polyclonal
35 antibody. Furthermore, the antibody specifically binds to at least one CS194 antigen

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selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

Another method is provided which detects antibodies which specifically bind to CS194 antigen in a test sample suspected of containing these antibodies. The method
5 comprises contacting the test sample with a polypeptide which contains at least one CS194 epitope, wherein the CS194 epitope comprises an amino acid sequence having at least 50% identity with an amino acid sequence encoded by a CS194 polynucleotide, or a fragment thereof. Contacting is carried out for a time and under conditions sufficient to allow antigen/antibody complexes to form. The method further entails
10 detecting complexes which contain the polypeptide. The polypeptide can be attached to a solid phase. Further, the polypeptide can be a recombinant protein or a synthetic peptide having at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

The present invention provides a cell transfected with a CS194 nucleic acid
15 sequence that encodes at least one epitope of a CS194 antigen, or fragment thereof. The nucleic acid sequence is selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof.

A method for producing antibodies to CS194 antigen also is provided, which method comprises administering to an individual an isolated immunogenic polypeptide
20 or fragment thereof, wherein the isolated immunogenic polypeptide comprises at least one CS194 epitope. The immunogenic polypeptide or fragment thereof is administered in an amount sufficient to produce an immune response. The isolated, immunogenic polypeptide comprises an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

Another method for producing antibodies which specifically bind to CS194
25 antigen is disclosed, which method comprises administering to an individual a plasmid comprising a nucleic acid sequence which encodes at least one CS194 epitope derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. The plasmid is administered in an amount such
30 that the plasmid is taken up by cells in the individual and expressed at levels sufficient to produce an immune response.

Also provided is a composition of matter that comprises a CS194 polynucleotide of at least about 10-12 nucleotides having at least 50% identity with a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or
35 complements thereof. The CS194 polynucleotide encodes an amino acid sequence having at least one CS194 epitope. Another composition of matter provided by the

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present invention comprises a polypeptide with at least one CS194 epitope of about 8-10 amino acids. The polypeptide comprises an amino acid sequence having at least 60% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. Also provided is a gene, or a
5 fragment thereof, coding for a CS194 polypeptide which has at least 60% identity with SEQUENCE ID NO 41; and a gene, or a fragment thereof, comprising DNA having at least 50% identity with SEQUENCE ID NO 18, SEQUENCE ID NO 19 or SEQUENCE ID NO 20.

10 Brief Description of the Drawings

Figures 1A-G show the nucleotide alignment of clones 1737775 (SEQUENCE ID NO 1), 776455 (SEQUENCE ID NO 2), 3234527 (SEQUENCE ID NO 3), 1628724 (SEQUENCE ID NO 4), 1427224 (SEQUENCE ID NO 5), 3235571 (SEQUENCE ID NO 6), 2680592 (SEQUENCE ID NO 7), 608819 (SEQUENCE ID
15 NO 8), 1629002 (SEQUENCE ID NO 9), 1626435 (SEQUENCE ID NO 10), 1582693 (SEQUENCE ID NO 11), 1281379 (SEQUENCE ID NO 12), 1628509 (SEQUENCE ID NO 13), 2060739 (SEQUENCE ID NO 14), 1624934 (SEQUENCE ID NO 15), 1800229 (SEQUENCE ID NO 16), 990829 (SEQUENCE ID NO 17; the full-length sequences of clone 1737775 (designated as clone 1737775IH (SEQUENCE
20 ID NO 18)) and clone 608819 (designated as clone 608819IH (SEQUENCE ID NO 19)); and the consensus sequence (SEQUENCE ID NO 20) derived therefrom.

Figure 2 shows the contig map depicting the formation of the consensus nucleotide sequence (SEQUENCE ID NO 20) from the nucleotide alignment of overlapping clones 1737775 (SEQUENCE ID NO 1), 776455 (SEQUENCE ID NO 2),
25 3234527 (SEQUENCE ID NO 3), 1628724 (SEQUENCE ID NO 4), 1427224 (SEQUENCE ID NO 5), 3235571 (SEQUENCE ID NO 6), 2680592 (SEQUENCE ID NO 7), 608819 (SEQUENCE ID NO 8), 1629002 (SEQUENCE ID NO 9), 1626435 (SEQUENCE ID NO 10), 1582693 (SEQUENCE ID NO 11), 1281379 (SEQUENCE ID NO 12), 1628509 (SEQUENCE ID NO 13), 2060739 (SEQUENCE ID NO 14),
30 1624934 (SEQUENCE ID NO 15), 1800229 (SEQUENCE ID NO 16), 990829 (SEQUENCE ID NO 17), 1737775IH (SEQUENCE ID NO 18) and 608819IH (SEQUENCE ID NO 19).

Figure 3 shows the results of the Western blot performed on a panel of tissue extracts using antiserum against a CS194 synthetic peptide (SEQUENCE ID NO 48).

Detailed Description of the Invention

The present invention provides a gene, or a fragment thereof, which codes for a CS194 polypeptide having at least about 60% identity with SEQUENCE ID NO 41.

The present invention further encompasses a CS194 gene, or a fragment thereof,
5 comprising DNA which has at least about 50% identity with SEQUENCE ID NO 18, SEQUENCE ID NO 19, or SEQUENCE ID NO 20.

The present invention also provides methods for assaying a test sample for products of a gastrointestinal tract (GI tract) tissue gene designated as CS194, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an
10 indication of the presence of GI tract tissue gene CS194. The method may include an amplification step, wherein one or more portions of the mRNA from CS194 corresponding to the gene or fragments thereof, is amplified. Methods also are provided for assaying for the translation products of CS194. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and
15 secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain mRNA sequences in test samples. Also disclosed are
20 nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, as targets for pharmaceutical screening assays and/or as components or as target sites for various therapies. Monoclonal and polyclonal antibodies directed against at least one epitope contained within these polypeptide sequences are useful as delivery agents for
25 therapeutic agents as well as for diagnostic tests and for screening for diseases or conditions associated with CS194, especially GI tract cancer. Isolation of sequences of other portions of the gene of interest can be accomplished utilizing probes or PCR primers derived from these nucleic acid sequences. This allows additional probes of the mRNA or cDNA of interest to be established, as well as corresponding encoded
30 polypeptide sequences. These additional molecules are useful in detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases and conditions of the GI tract, such as GI tract cancer, characterized by CS194, as disclosed herein.

Techniques for determining amino acid sequence "similarity" are well-known in
35 the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or

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possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide
5 sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more polynucleotide sequences can be compared by determining
10 their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The programs available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI), for example, the GAP program, are capable of calculating both the identity between two polynucleotides and the identity and similarity between two
15 polypeptide sequences, respectively. Other programs for calculating identity or similarity between sequences are known in the art.

The compositions and methods described herein will enable the identification of certain markers as indicative of a GI tract tissue disease or condition; the information obtained therefrom will aid in the detecting, diagnosing, staging, monitoring,
20 prognosticating, preventing or treating, or determining diseases or conditions associated with CS194, especially GI tract cancer. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), and hybridization. In addition, the nucleotide sequences
25 provided herein contain open reading frames from which an immunogenic epitope may be found. This epitope is believed to be unique to the disease state or condition associated with CS194. It also is thought that the polynucleotides or polypeptides and protein encoded by the CS194 gene are useful as a marker. This marker is either elevated in disease such as GI tract cancer, altered in disease such as GI tract cancer, or
30 present as a normal protein but appearing in an inappropriate body compartment. The uniqueness of the epitope may be determined by (i) its immunological reactivity and specificity with antibodies directed against proteins and polypeptides encoded by the CS194 gene, and (ii) its nonreactivity with any other tissue markers. Methods for determining immunological reactivity are well-known and include, but are not limited
35 to, for example, radioimmunoassay (RIA), enzyme-linked immunoabsorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA),

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chemiluminescent immunoassay (CLIA) and others. Several examples of suitable methods are described herein.

Unless otherwise stated, the following terms shall have the following meanings:

5 A polynucleotide "derived from" or "specific for" a designated sequence refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary or identical to a sequence
10 which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to determine the uniqueness of a designated sequence. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

15 The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including, but not limited to, chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either
20 a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with the intended use.

A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides,
25 preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the specified nucleotide sequence.

The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target
30 nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., PNA as defined hereinbelow) which can be used to identify a specific
35 polynucleotide present in samples bearing the complementary sequence.

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“Encoded by” refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence. Thus, a “polypeptide,” “protein,” or “amino acid” sequence has at least about 50% identity, preferably about 60% identity, more preferably about 75-85% identity, and most preferably about 90-95% or more identity to a CS194 amino acid sequence. Further, the CS194 “polypeptide,” “protein,” or “amino acid” sequence may have at least about 60% similarity, preferably at least about 75% similarity, more preferably about 85% similarity, and most preferably about 95% or more similarity to a polypeptide or amino acid sequence of CS194. This amino acid sequence can be selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

A “recombinant polypeptide,” “recombinant protein,” or “a polypeptide produced by recombinant techniques,” which terms may be used interchangeably herein, describes a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it is linked in nature. A recombinant or encoded polypeptide or protein is not necessarily translated from a designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term “synthetic peptide” as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to the routineer. These synthetic peptides are useful in various applications.

The term “polynucleotide” as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modifications, such as methylation or capping and unmodified forms of the polynucleotide. The terms “polynucleotide,” “oligomer,” “oligonucleotide,” and “oligo” are used interchangeably herein.

“A sequence corresponding to a cDNA” means that the sequence contains a polynucleotide sequence that is identical or complementary to a sequence in the designated DNA. The degree (or “percent”) of identity or complementarity to the cDNA will be approximately 50% or greater, preferably at least about 70% or greater, and

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more preferably at least about 90% or greater. The sequence that corresponds to the identified cDNA will be at least about 50 nucleotides in length, preferably at least about 60 nucleotides in length, and more preferably at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

“Purified polynucleotide” refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

“Purified polypeptide” or “purified protein” means a polypeptide of interest or fragment thereof which is essentially free of, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, cellular components with which the polypeptide of interest is naturally associated. Methods for purifying polypeptides of interest are known in the art.

The term “isolated” means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

“Polypeptide” and “protein” are used interchangeably herein and indicate at least one molecular chain of amino acids linked through covalent and/or non-covalent bonds. The terms do not refer to a specific length of the product. Thus peptides, oligopeptides and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

A "fragment" of a specified polypeptide refers to an amino acid sequence which comprises at least about 3-5 amino acids, more preferably at least about 8-10 amino acids, and even more preferably at least about 15-20 amino acids derived from the specified polypeptide.

5 "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original progeny of the original cell which has been transfected.

10 As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

15 The term "control sequence" refers to a polynucleotide sequence which is necessary to effect the expression of a coding sequence to which it is ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include a promoter, a ribosomal binding site and terminators; in eukaryotes, such control sequences generally include promoters, 20 terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

25 "Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequence.

30 The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide. This region may represent a portion of a coding sequence or a total coding sequence.

35 A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -

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terminus. A coding sequence can include, but is not limited to, mRNA, cDNA and recombinant polynucleotide sequences.

5 The term “immunologically identifiable with/as” refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to the routineer and also are described herein. The uniqueness of an epitope also can be determined by computer searches of known data banks, such as GenBank, for the polynucleotide sequence which encodes the epitope and by amino acid sequence comparisons with other known proteins.

10 As used herein, “epitope” means an antigenic determinant of a polypeptide or protein. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

15 A “conformational epitope” is an epitope that is comprised of a specific juxtaposition of amino acids in an immunologically recognizable structure, such amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

20 A polypeptide is “immunologically reactive” with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly, by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

25 As used herein, the term “immunogenic polypeptide containing an epitope of interest” means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

30 The term “transfection” refers to the introduction of an exogenous polynucleotide into a prokaryotic or eucaryotic host cell, irrespective of the method used for the introduction. The term “transfection” refers to both stable and transient introduction of the polynucleotide, and encompasses direct uptake of polynucleotides, transformation, transduction, and f-mating. Once introduced into the host cell, the

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exogenous polynucleotide may be maintained as a non-integrated replicon, for example, a plasmid, or alternatively, may be integrated into the host genome.

“Treatment” refers to prophylaxis and/or therapy.

5 The term “individual” as used herein refers to vertebrates, particularly members of the mammalian species and includes, but is not limited to, domestic animals, sports animals, primates and humans; more particularly, the term refers to humans.

The term “sense strand” or “plus strand” (or “+”) as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term “antisense strand” or “minus strand” (or “-”) denotes a nucleic acid that contains a
10 sequence that is complementary to that of the “plus” strand.

The term “test sample” refers to a component of an individual's body which is the source of the analyte (such as antibodies of interest or antigens of interest). These components are well known in the art. A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well
15 known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained thereby to release target nucleic acids. These test samples include biological samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, sputum, bronchial washing, bronchial aspirates,
20 urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; tissue specimens which may be fixed; and cell specimens which may be fixed.

“Purified product” refers to a preparation of the product which has been isolated
25 from the cellular constituents with which the product is normally associated and from other types of cells which may be present in the sample of interest.

“PNA” denotes a “peptide nucleic acid analog” which may be utilized in a procedure such as an assay described herein to determine the presence of a target.

“MA” denotes a “morpholino analog” which may be utilized in a procedure such as an
30 assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include
35 manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods

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physical means, specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors, and enzymes and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal and complexes thereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent," as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. In addition to being an antibody member of a specific binding pair, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to the polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazole or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromagens, catalysts such as enzymes, luminescent compounds such as fluorescein

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and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase and the like. The selection of a particular label is not critical, but it must be capable of producing a signal either by itself or in conjunction with one or more additional substances.

“Solid phases” (“solid supports”) are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic or non-magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells and Duracytes® (red blood cells “fixed” by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The “solid phase” is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A “solid phase,” as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent.

Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens.

Microporous structures generally are preferred, but materials with a gel structure in the

hydrated state may be used as well. Such useful solid supports include, but are not limited to, nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore size may vary within wide limits and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

Reagents.

The present invention provides reagents such as polynucleotide sequences derived from a GI tract tissue of interest and designated as CS194, polypeptides encoded thereby and antibodies specific for these polypeptides. The present invention also provides reagents such as oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. The polynucleotides, polypeptides, or antibodies of the present invention may be used to provide information leading to the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating of, or determining the predisposition to, diseases and conditions of the GI tract, such as GI tract cancer. The sequences disclosed herein represent unique polynucleotides which can be used in assays or for producing a specific profile of gene transcription activity. Such assays are disclosed in European Patent Number 0373203B1 and International Publication No. WO 95/11995, which are hereby incorporated by reference.

Selected CS194-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression. Such methods may employ CS194 polynucleotides or oligonucleotides, fragments or derivatives thereof, or nucleic acid sequences complementary thereto.

The polynucleotides disclosed herein, their complementary sequences, or fragments of either, can be used in assays to detect, amplify or quantify genes, nucleic acids, cDNAs or mRNAs relating to GI tract tissue disease and conditions associated therewith. They also can be used to identify an entire or partial coding region of a CS194 polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide may be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and an additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally an additional coding sequence) and non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

In addition, the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional 5' amino acid residues. A protein having a prosequence is a proprotein and may, in some cases, be an inactive form of the protein. Once the prosequence is cleaved, an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence, or for a protein having both a prosequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. a COS-7 cell line, is used. The HA tag

corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson et al., Cell 37:767 (1984).

It is contemplated that polynucleotides will be considered to hybridize to the sequences provided herein if there is at least 50%, preferably at least 70%, and more preferably at least 90% identity between the polynucleotide and the sequence.

The present invention also provides an antibody produced by using a purified CS194 polypeptide of which at least a portion of the polypeptide is encoded by a CS194 polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of CS194 antigen in test samples. The presence of CS194 antigen in the test samples is indicative of the presence of a GI tract disease or condition. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of CS194 polypeptide in conditions associated with altered or abnormal expression.

The present invention further relates to a CS194 polypeptide which has the deduced amino acid sequence as provided herein, as well as fragments, analogs and derivatives of such polypeptide. The polypeptide of the present invention may be a recombinant polypeptide, a natural purified polypeptide or a synthetic polypeptide. The fragment, derivative or analog of the CS194 polypeptide may be one in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present invention. The polypeptides and polynucleotides of the present invention are provided preferably in an isolated form and preferably purified.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or threonine with serine. In contrast, variations may include

nonconservative changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison WI).

Probes constructed according to the polynucleotide sequences of the present invention can be used in various assay methods to provide various types of analysis. For example, such probes can be used in fluorescent in situ hybridization (FISH) technology to perform chromosomal analysis, and used to identify cancer-specific structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotides probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly- detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in tissue specimens or cells.

This invention also provides teachings as to the production of the polynucleotides and polypeptides provided herein.

Probe Assays

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multi-gene family or in related species like mouse and man.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically

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Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described by J.C. Guatelli et al., PNAS USA 87:1874-1878 (1990) and also described by J. Compton, Nature 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G.T. Walker et al., Clin. Chem. 42:9-13 [1996]) and European Patent Application No. 684315; and target mediated amplification, as described in International Publication No. WO 93/22461.

Detection of CS194 may be accomplished using any suitable detection method, including those detection methods which are currently well known in the art, as well as detection strategies which may evolve later. Examples of the foregoing presently known detection methods are hereby incorporated herein by reference. See, for
5 example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. Examples of such detection methods include target amplification methods as well as signal amplification technologies. An example of presently known detection methods would include the nucleic acid amplification technologies referred to as PCR, LCR, NASBA, SDA, RCR and TMA. See, for example, Caskey et al., U.S. Patent
10 No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. All of the foregoing are hereby incorporated by reference. Detection may also be accomplished using signal amplification such as that disclosed in Snitman et al., U.S. Patent No. 5,273,882. While the amplification of target or signal is preferred at present, it is contemplated and within the scope of the present invention that ultrasensitive detection methods which do
15 not require amplification can be utilized herein.

Detection, both amplified and non-amplified, may be (combined) carried out using a variety of heterogeneous and homogeneous detection formats. Examples of heterogeneous detection formats are disclosed in Snitman et al., U.S. Patent No. 5,273,882, Albarella et al in EP-84114441.9, Urdea et al., U.S. Patent No.
20 5,124,246, Ullman et al. U.S. Patent No. 5,185,243 and Kourilsky et al., U.S. Patent No. 4,581,333. All of the foregoing are hereby incorporated by reference. Examples of homogeneous detection formats are disclosed in, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015, which are incorporated herein by reference. Also contemplated and within the scope of the present invention is the
25 use of multiple probes in the hybridization assay, which use improves sensitivity and amplification of the CS194 signal. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015, which are incorporated herein by reference.

In one embodiment, the present invention generally comprises the steps of
30 contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method provided herein are labeled with capture and detection labels, wherein probes are labeled with one type of label and primers are
35 labeled with another type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences.

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enzymes previously mentioned, a nucleic acid amplification reaction mixture may also comprise other reagents which are well known and include but are not limited to: enzyme cofactors such as manganese; magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as, for example,
5 deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

While the amplification primers initiate amplification of the target sequence, the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for
10 example, peptide nucleic acids which are disclosed in International Publication No. WO 92/20702; morpholino analogs which are described in U.S. Patents Nos 5,185,444, 5,034,506 and 5,142,047; and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence
15 and therefore may have to be rendered "non-extendible" in that additional dNTPs cannot be added to the probe. In and of themselves, analogs usually are non-extendible and nucleic acid probes can be rendered non-extendible by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection
20 label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 and incorporated herein by reference describes modifications which can be used to render a probe non-extendible.

The ratio of primers to probes is not important. Thus, either the probes or
25 primers can be added to the reaction mixture in excess whereby the concentration of one would be greater than the concentration of the other. Alternatively, primers and probes can be employed in equivalent concentrations. Preferably, however, the primers are added to the reaction mixture in excess of the probes. Thus, primer to probe ratios of, for example, 5:1 and 20:1, are preferred.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences.
30 Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the
35 range of between 10 and 25 nucleotides long.

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Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is a matter of routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), DuPont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention. Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications U.S. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, which are each incorporated herein by reference, teach methods for labeling probes at their 5' and 3' termini, respectively. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J.S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

A capture label is attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

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Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous immunoassays. Preferably, in this embodiment, detection is performed according to the protocols used by the commercially available Abbott LCx[®] instrumentation (Abbott Laboratories, 5 Abbott Park, IL).

The primers and probes disclosed herein are useful in typical PCR assays, wherein the test sample is contacted with a pair of primers, amplification is performed, the hybridization probe is added, and detection is performed.

Another method provided by the present invention comprises contacting a test 10 sample with a plurality of polynucleotides, wherein at least one polynucleotide is a CS194 molecule as described herein, hybridizing the test sample with the plurality of polynucleotides and detecting hybridization complexes. Hybridization complexes are identified and quantitated to compile a profile which is indicative of GI tract tissue disease, such as GI tract cancer. Expressed RNA sequences may further be detected by 15 reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

Drug Screening and Gene Therapy.

The present invention also encompasses the use of gene therapy methods for the introduction of anti-sense CS194 derived molecules, such as polynucleotides or 20 oligonucleotides of the present invention, into patients with conditions associated with abnormal expression of polynucleotides related to a GI tract tissue disease or condition especially GI tract cancer. These molecules, including antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of CS194-mRNA, and may be used therapeutically in the treatment of conditions associated with altered or 25 abnormal expression of CS194 polynucleotide.

Alternatively, the oligonucleotides described above can be delivered to cells by procedures known in the art such that the anti-sense RNA or DNA may be expressed in vivo to inhibit production of a CS194 polypeptide in the manner described above. Antisense constructs to a CS194 polynucleotide, therefore, reverse the action of CS194 30 transcripts and may be used for treating GI tract tissue disease conditions, such as GI tract cancer. These antisense constructs may also be used to treat tumor metastases.

The present invention also provides a method of screening a plurality of compounds for specific binding to CS194 polypeptide(s), or any fragment thereof, to identify at least one compound which specifically binds the CS194 polypeptide. Such a 35 method comprises the steps of providing at least one compound; combining the CS194

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polypeptide with each compound under suitable conditions for a time sufficient to allow binding; and detecting the CS194 polypeptide binding to each compound.

The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly.

5 One method of screening utilizes eukaryotic or prokaryotic host cells which are stably transfected with recombinant nucleic acids which can express the polypeptide or peptide fragment. A drug, compound, or any other agent may be screened against such transfected cells in competitive binding assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in either
10 viable or fixed cells.

The present invention thus provides methods of screening for drugs, compounds, or any other agent which can be used to treat diseases associated with CS194. These methods comprise contacting the agent with a polypeptide or fragment thereof and assaying for either the presence of a complex between the agent and the
15 polypeptide, or for the presence of a complex between the polypeptide and the cell. In competitive binding assays, the polypeptide typically is labeled. After suitable incubation, free (or uncomplexed) polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is used as a measure of the ability of the particular agent to bind to the polypeptide or to interfere
20 with the polypeptide/cell complex.

The present invention also encompasses the use of competitive screening assays in which neutralizing antibodies capable of binding polypeptide specifically compete with a test agent for binding to the polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any polypeptide in the test sample
25 which shares one or more antigenic determinants with a CS194 polypeptide as provided herein.

Another technique for screening provides high throughput screening for compounds having suitable binding affinity to at least one polypeptide of CS194 disclosed herein. Briefly, large numbers of different small peptide test compounds are
30 synthesized on a solid phase, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptide and washed. Polypeptide thus bound to the solid phase is detected by methods well-known in the art. Purified polypeptide can also be coated directly onto plates for use in the screening techniques described herein. In addition, non-neutralizing antibodies can be used to capture the polypeptide and
35 immobilize it on the solid support. See, for example, EP 84/03564, published on September 13, 1984, which is incorporated herein by reference.

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The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of the small molecules including agonists, antagonists, or inhibitors with which they interact. Such structural analogs can be used to design drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide *in vivo*. J. Hodgson, Bio/Technology 9:19-21 (1991), incorporated herein by reference.

For example, in one approach, the three-dimensional structure of a polypeptide, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors

Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton et al., Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S.B.P. Athauda et al., J Biochem. (Tokyo) 113 (6):742-746 (1993), incorporated herein by reference.

It also is possible to isolate a target-specific antibody selected by an assay as described hereinabove, and then to determine its crystal structure. In principle this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then can be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

A sufficient amount of a recombinant polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which is derivable from the nucleic acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of, or in addition to, x-ray crystallography.

Antibodies specific to a CS194 polypeptide (e.g., anti-CS194 antibodies) further may be used to inhibit the biological action of the polypeptide by binding to the

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polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat GI tract tissue diseases including GI tract cancer and its metastases.

Further, such antibodies can detect the presence or absence of a CS194 polypeptide in a test sample and, therefore, are useful as diagnostic markers for the diagnosis of a GI tract tissue disease or condition especially GI tract cancer. Such antibodies may also function as a diagnostic marker for GI tract tissue disease conditions, such as GI tract cancer.

The present invention also is directed to antagonists and inhibitors of the polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example, could be an antibody against the polypeptide which eliminates the activity of a CS194 polypeptide by binding a CS194 polypeptide, or in some cases the antagonist may be an oligonucleotide. Examples of small molecule inhibitors include, but are not limited to, small peptides or peptide-like molecules.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier including, but not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. Administration of CS194 polypeptide inhibitors is preferably systemic. The present invention also provides an antibody which inhibits the action of such a polypeptide.

Antisense technology can be used to reduce gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of the CS194 polypeptide. For triple helix, see, for example, Lee et al, Nuc. Acids Res. 6:3073 (1979); Cooney et al, Science 241:456 (1988); and Dervan et al, Science 251:1360 (1991) The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of a mRNA molecule into the CS194 polypeptide. For antisense, see, for example, Okano, J. Neurochem. 56:560 (1991); and "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression," CRC Press, Boca Raton, Fla. (1988). Antisense oligonucleotides act with greater efficacy when modified to contain artificial internucleotide linkages which render the molecule resistant to nucleolytic cleavage.

Recombinant Technology.

The present invention also provides vectors which include CS194
10 polynucleotides of the present invention, host cells which are genetically engineered
with vectors of the present invention and the production of polypeptides of the present
invention by recombinant techniques.

The polynucleotides of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, the polynucleotide sequence may be included in any one of a variety of expression vehicles, in particular, vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus and pseudorabies. However, any other plasmid or vector may be used so long as it is replicable and viable in the host.

30 The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction endonuclease sites by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art. The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s)
35 (promoter) to direct mRNA synthesis. Representative examples of such promoters include, but are not limited to, the LTR or the SV40 promoter, the *E. coli* lac or trp, the

phage lambda P sub L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transfected host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transfect an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; Streptomyces sp.; fungal cells, such as yeast; insect cells, such as Drosophila and Sf9; animal cells, such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available. The following vectors are provided by way of example. Bacterial: pINCY (Incyte Pharmaceuticals Inc., Palo Alto, CA), pSPORT1 (Life Technologies, Gaithersburg, MD), pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Plasmid pINCY is generally identical to the plasmid pSPORT1 (available from Life Technologies, Gaithersburg, MD) with the exception that it has two modifications in the polylinker (multiple cloning site). These modifications are (1) it lacks a HindIII restriction site and (2) its EcoRI restriction site lies at a different location. pINCY is created from pSPORT1 by cleaving pSPORT1 with both HindIII and EcoRI and

Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor, NY, 1989), which is hereby incorporated by reference.

Transcription of a DNA encoding the polypeptide(s) of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector.

5 Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin and adenovirus enhancers.

10 Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transfection of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-
15 phosphoglycerate kinase (PGK), alpha factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a
20 fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation
25 initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transfection include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera
30 Pseudomonas, Streptomyces and Staphylococcus, although others may also be employed as a routine matter of choice.

Useful expression vectors for bacterial use comprise a selectable marker and bacterial origin of replication derived from plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Other vectors include but are not
35 limited to PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1

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(Promega Biotec, Madison, WI). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transfection of a suitable host and growth of the host to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well-known to the ordinary artisan.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, such as the C127, HEK-293, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Representative, useful vectors include pRc/CMV and pcDNA3 (available from Invitrogen, San Diego, CA).

CS194 polypeptides are recovered and purified from recombinant cell cultures by known methods including affinity chromatography, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, et al., J. Biol. Chem. 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Thus, polypeptides of the present invention may be naturally purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure,

the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue.

5 The starting plasmids can be constructed from available plasmids in accord with published, known procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to one of ordinary skill in the art.

10 The following is the general procedure for the isolation and analysis of cDNA clones. In a particular embodiment disclosed herein, mRNA was isolated from GI tract tissue and used to generate the cDNA library. GI tract tissue was obtained from patients by surgical resection and was classified as tumor or non-tumor tissue by a pathologist.

15 The cDNA inserts from random isolates of the GI tract tissue libraries were sequenced in part, analyzed in detail as set forth in the Examples and are disclosed in the Sequence Listing as SEQUENCE ID NOS 1-17. Also analyzed in detail as set forth in the Examples, and disclosed in the Sequence Listing, are the full-length sequences of clones 1737775 and 608819 (referred to as clones 1737775IH and 608819IH (SEQUENCE ID NOS 18 and 19, respectively)). The consensus sequence of these inserts is presented as SEQUENCE ID NO 20. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred and sometimes several thousand bases in length and, with current technology, cannot be cloned in their entirety because of vector limitations, incomplete reverse transcription of the first strand, or incomplete replication of the second strand. Contiguous, secondary clones containing additional nucleotide sequences may be obtained using a variety of methods known to those of skill in the art.

20 Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase, Klenow fragment, Sequenase (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded templates. The chain termination reaction products may be electrophoresed on urea/polyacrylamide gels and detected either by autoradiography (for radionucleotide labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day

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using machines such as the Applied Biosystems 377 DNA Sequencers (Applied Biosystems, Foster City, CA).

The reading frame of the nucleotide sequence can be ascertained by several types of analyses. First, reading frames contained within the coding sequence can be analyzed for the presence of start codon ATG and stop codons TGA, TAA or TAG. Typically, one reading frame will continue throughout the major portion of a cDNA sequence while other reading frames tend to contain numerous stop codons. In such cases, reading frame determination is straightforward. In other more difficult cases, further analysis is required.

Algorithms have been created to analyze the occurrence of individual nucleotide bases at each putative codon triplet. See, for example J.W. Fickett, Nuc. Acids Res. 10:5303 (1982). Coding DNA for particular organisms (bacteria, plants and animals) tends to contain certain nucleotides within certain triplet periodicities, such as a significant preference for pyrimidines in the third codon position. These preferences have been incorporated into widely available software which can be used to determine coding potential (and frame) of a given stretch of DNA. The algorithm-derived information combined with start/stop codon information can be used to determine proper frame with a high degree of certainty. This, in turn, readily permits cloning of the sequence in the correct reading frame into appropriate expression vectors.

The nucleic acid sequences disclosed herein may be joined to a variety of other polynucleotide sequences and vectors of interest by means of well-established recombinant DNA techniques. See J. Sambrook et al., supra. Vectors of interest include cloning vectors, such as plasmids, cosmids, phage derivatives, phagemids, as well as sequencing, replication and expression vectors, and the like. In general, such vectors contain an origin of replication functional in at least one organism, convenient restriction endonuclease digestion sites and selectable markers appropriate for particular host cells. The vectors can be transferred by a variety of means known to those of skill in the art into suitable host cells which then produce the desired DNA, RNA or polypeptides.

Occasionally, sequencing or random reverse transcription errors will mask the presence of the appropriate open reading frame or regulatory element. In such cases, it is possible to determine the correct reading frame by attempting to express the polypeptide and determining the amino acid sequence by standard peptide mapping and sequencing techniques. See, F.M. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY (1989). Additionally, the actual reading frame of a given nucleotide sequence may be determined by transfection of host cells

with vectors containing all three potential reading frames. Only those cells with the nucleotide sequence in the correct reading frame will produce a peptide of the predicted length.

The nucleotide sequences provided herein have been prepared by current, state-of-the-art, automated methods and, as such, may contain unidentified nucleotides. These will not present a problem to those skilled in the art who wish to practice the invention. Several methods employing standard recombinant techniques, described in J. Sambrook (supra) or periodic updates thereof, may be used to complete the missing sequence information. The same techniques used for obtaining a full length sequence, as described herein, may be used to obtain nucleotide sequences.

Expression of a particular cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting this vector into an appropriate expression host. The cloning vector used for the generation of the GI tract tissue cDNA library can be used for transcribing mRNA of a particular cDNA and contains a promoter for beta-galactosidase, an amino-terminal met and the subsequent seven amino acid residues of beta-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription, as well as a number of unique restriction sites, including EcoRI, for cloning. The vector can be transfected into an appropriate host strain of E. coli.

Induction of the isolated bacterial strain with isopropylthiogalactoside (IPTG) using standard methods will produce a fusion protein which contains the first seven residues of beta-galactosidase, about 15 residues of linker and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, the correct frame can be obtained by deletion or insertion of an appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide primers containing cloning sites and segments of DNA sufficient to hybridize to stretches at both ends of the target cDNA can be synthesized chemically by standard methods. These primers can then be used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digestion of the cDNA with appropriate restriction enzymes and filling in the missing gene segments

with chemically synthesized oligonucleotides. Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include, but are not limited to, mammalian cells, such as Chinese Hamster Ovary (CHO) and human embryonic kidney (HEK) 293 cells, insect cells, such as Sf9 cells, yeast cells, such as Saccharomyces cerevisiae and bacteria, such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the beta-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker, such as the neomycin phosphotransferase gene, to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require the addition of 3' poly A tail if the sequence of interest lacks poly A.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include, but are not limited to, MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts; or alpha factor, alcohol oxidase or PGH promoters for yeast. Adenoviral vectors with or without transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to drive protein expression in mammalian cell lines. Once homogeneous cultures of recombinant cells are obtained, large quantities of recombinantly produced protein can be recovered from the conditioned medium and analyzed using chromatographic methods well known in the art. An alternative method for the production of large amounts of secreted protein involves the transfection of mammalian embryos and the recovery of the recombinant protein from milk produced by transgenic cows, goats, sheep, etc. Polypeptides and closely related molecules may be expressed recombinantly in such a way as to facilitate protein purification. One approach involves expression of a chimeric protein which includes one or more additional polypeptide domains not naturally present on human polypeptides. Such purification-facilitating domains include, but are not limited to, metal-chelating peptides such as histidine-tryptophan domains that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

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Immunoassays.

CS194 polypeptides, including fragments, derivatives, and analogs thereof, or cells expressing such polypeptides, can be utilized in a variety of assays, many of which are described herein, for the detection of antibodies to GI tract tissue. They also
5 can be used as immunogens to produce antibodies. These antibodies can be, for example, polyclonal or monoclonal antibodies, chimeric, single chain and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

10 For example, antibodies generated against a polypeptide comprising a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, goat or human. A mouse, rabbit or goat is preferred. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. The antibody
15 so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies that bind the native polypeptide. Such antibodies then can be used to isolate the polypeptide from test samples such as tissue suspected of containing that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by
20 continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor et al, Immun. Today 4:72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole et al., in Monoclonal Antibodies and Cancer Therapy,
25 Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Patent No. 4,946,778, which is incorporated herein by reference.

Various assay formats may utilize the antibodies of the present invention,
30 including "sandwich" immunoassays and probe assays. For example, the antibodies of the present invention, or fragments thereof, can be employed in various assay systems to determine the presence, if any, of CS194 antigen in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted
35 with a test sample, to form a first mixture. This first mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator

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reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form
5 antibody/antigen/antibody complexes. The presence of CS194 antigen in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of CS194 antigen present in the test sample is proportional to the signal generated.

In an alternative assay format, a mixture is formed by contacting: (1) a
10 polyclonal antibody, monoclonal antibody, or fragment thereof, which specifically binds to CS194 antigen, or a combination of such antibodies bound to a solid support; (2) the test sample; and (3) an indicator reagent comprising a monoclonal antibody, polyclonal antibody, or fragment thereof, which specifically binds to a different CS194 antigen (or a combination of these antibodies) to which a signal generating compound is
15 attached. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of CS194 antigen present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of CS194 antigen present in the test sample is proportional to the signal generated.

20 In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to CS194 antigen. For example, CS194 polypeptides such as the recombinant antigens disclosed herein, either alone or in combination, are coated on a solid phase. A test sample suspected of containing antibody to CS194 antigen then is
25 incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively
30 measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of CS194 antigens in tissue sections, as well as in cells, by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein,
35 colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary labeled anti-species antibodies (with various labels as exemplified

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herein) to track the histopathology of disease also are within the scope of the present invention.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific CS194 polypeptides from cell cultures or biological tissues such as to purify recombinant and native CS194 proteins.

The monoclonal antibodies of the invention also can be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect CS194 antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one CS194 antibody of the invention, along with antibodies which specifically bind to other CS194 regions, each antibody having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to CS194 polypeptides disclosed herein and other monoclonal antibodies specific to other antigenic determinants of CS194 antigens or other related proteins.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a CS194 polypeptide or other CS194 polypeptides additionally used in the assay. The polyclonal antibody used preferably is of mammalian origin such as, human, goat, rabbit or sheep polyclonal antibody which binds CS194 polypeptide. Most preferably, the polyclonal antibody is of rabbit origin. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different binding specificity to CS194 polypeptides, they are useful for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to, diseases and conditions of the GI tract, such as GI tract cancer.

It is contemplated and within the scope of the present invention that CS194 antigen may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which peptide comprises an amino acid sequence of CS194. The amino acid sequence of such a polypeptide is selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides, identifying different epitopes of CS194, can be used in combination in an assay for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or

determining the predisposition to diseases and conditions of the GI tract, such as GI tract cancer. In this case, all of these peptides can be coated onto one solid phase; or each separate peptide may be coated onto separate solid phases, such as microparticles, and then combined to form a mixture of peptides which can be later used in assays.

- 5 Furthermore, it is contemplated that multiple peptides which define epitopes from different antigens may be used for the detection, diagnosis, staging, monitoring, prognosis, prevention or treatment of, or determining the predisposition to, diseases and conditions of the GI tract, such as GI tract cancer. Peptides coated on solid phases or labeled with detectable labels are then allowed to compete with those present in a
- 10 patient sample (if any) for a limited amount of antibody. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of CS194 antigen in the patient sample. The presence of CS194 antigen indicates the presence of GI tract tissue disease, especially GI tract cancer, in the patient. Variations of assay formats are known to those of ordinary skill
- 15 in the art and many are discussed herein below.

- In another assay format, the presence of anti-CS194 antibody and/or CS194 antigen can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent comprises a first binding member specific for a first analyte attached to a solid
- 20 phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent
- 25 comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture
- 30 reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from the expression systems disclosed herein may be utilized, as well as monoclonal antibodies
- 35 produced from the proteins derived from the expression systems as disclosed herein.

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For example, in this assay system, CS194 antigen can be the first analyte. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of antibody against CS194 antigen in test samples. For example, a test sample is incubated with a solid phase to which at least one polypeptide such as a recombinant protein or synthetic peptide has been attached. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen.

In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached, and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of antibody against CS194 antigen. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared

solid phase, and following standard incubation and washing steps as deemed or required, a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent which subsequently is detected. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for CS194 produced or derived from a first source as the capture antigen and an antigen specific for CS194 from a different second source is contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership and is incorporated herein by reference.

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer (described in EP publication 0326100 and EP publication No. 0406473), can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EPO Publication No. 0 273,115.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described in, for example, published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, particularly in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding

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untrained personnel and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

E. coli bacteria (clones 1737775 and 608819) were deposited with the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 20852, on 9/12/97 and 6/25/97, respectively. The deposits were under the terms of the Budapest Treaty and will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable period of the U.S. patent, whichever is longer. The deposits and any other deposited material described herein are provided for convenience only, and are not required to practice the present invention in view of the teachings provided herein. The cDNA sequence in all of the deposited material is incorporated herein by reference. Clone 1737775 was accorded A.T.C.C. Deposit No. 98542, while clone 608819 was accorded A.T.C.C. Deposit No. 98460.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the scope of the present invention.

EXAMPLES

Example 1: Identification of Gastrointestinal Tract Tissue

Library CS194 Gene-Specific Clones

A. Library Comparison of Expressed Sequence Tags (ESTs) or Transcript Images. Partial sequences of cDNA clone inserts, so-called "expressed sequence tags" (ESTs), were derived from cDNA libraries made from GI tract tumor tissues, GI tract non-tumor tissues and numerous other tissues, both tumor and non-tumor and entered into a database (LIFESEQ™ database, available from Incyte Pharmaceuticals, Palo Alto, CA) as gene transcript images. See International Publication No. WO 95/20681. (A transcript image is a listing of the number of EST's for each of the represented genes in a given tissue library. ESTs sharing regions of mutual sequence overlap are classified into clusters. A cluster is assigned a clone number from a representative 5' EST. Often, a cluster of interest can be extended by comparing its consensus sequence with sequences of other EST's which did not meet the criteria for automated clustering. The alignment of all available clusters and single ESTs represent a contig from which a consensus sequence is derived.) The transcript images then were evaluated to identify EST sequences that were representative primarily of the GI tract tissue libraries. These target clones then were ranked according to their abundance (occurrence) in the target libraries and their absence from background libraries. Higher abundance clones with low background occurrence were given higher study priority. ESTs corresponding to

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the consensus sequence of CS194 were found in 41.4% (24 of 58) of GI tract tissue libraries. ESTs corresponding to the consensus sequence SEQUENCE ID NO 20 (or fragments thereof) were found in 0.4% (2 of 506) of the other, non-GI tract, libraries of the data base. Therefore, the consensus sequence or fragment thereof was found
5 more than 104 times more often in GI tract than non-GI tract tissues. Overlapping clones 1737775 (SEQUENCE ID NO 1), 776455 (SEQUENCE ID NO 2), 3234527 (SEQUENCE ID NO 3), 1628724 (SEQUENCE ID NO 4), 1427224 (SEQUENCE ID NO 5), 3235571 (SEQUENCE ID NO 6), 2680592 (SEQUENCE ID NO 7), 608819 (SEQUENCE ID NO 8), 1629002 (SEQUENCE ID NO 9), 1626435 (SEQUENCE ID
10 NO 10), 1582693 (SEQUENCE ID NO 11), 1281379 (SEQUENCE ID NO 12), 1628509 (SEQUENCE ID NO 13), 2060739 (SEQUENCE ID NO 14), 1624934 (SEQUENCE ID NO 15), 1800229 (SEQUENCE ID NO 16), and 990829 (SEQUENCE ID NO 17) were identified for further study. These represented the minimum number of clones that were needed to form the contig and from which, along
15 with the full-length sequence clones 1737775IH (SEQUENCE ID NO 18) and 608819IH (SEQUENCE ID NO 19), the consensus sequence provided herein (SEQUENCE ID NO 20) was derived.

B. Generation of a Consensus Sequence. The nucleotide sequences of clones 1737775 (SEQUENCE ID NO 1), 776455 (SEQUENCE ID NO 2), 3234527
20 (SEQUENCE ID NO 3), 1628724 (SEQUENCE ID NO 4), 1427224 (SEQUENCE ID NO 5), 3235571 (SEQUENCE ID NO 6), 2680592 (SEQUENCE ID NO 7), 608819 (SEQUENCE ID NO 8), 1629002 (SEQUENCE ID NO 9), 1626435 (SEQUENCE ID NO 10), 1582693 (SEQUENCE ID NO 11), 1281379 (SEQUENCE ID NO 12), 1628509 (SEQUENCE ID NO 13), 2060739 (SEQUENCE ID NO 14), 1624934
25 (SEQUENCE ID NO 15), 1800229 (SEQUENCE ID NO 16), 990829 (SEQUENCE ID NO 17), 1737775IH (SEQUENCE ID NO 18), and 608819IH (SEQUENCE ID NO 19), were entered in the Sequencher™ Program (available from Gene Codes Corporation, Ann Arbor, MI) in order to generate a nucleotide alignment (contig map) and then generate their consensus sequence (SEQUENCE ID NO 20). Figures 1A-G
30 show the nucleotide sequence alignment of these clones and their resultant nucleotide consensus sequence (SEQUENCE ID NO 20). Figure 2 presents the contig map depicting the clones, 1737775 (SEQUENCE ID NO 1), 776455 (SEQUENCE ID NO 2), 3234527 (SEQUENCE ID NO 3), 1628724 (SEQUENCE ID NO 4), 1427224 (SEQUENCE ID NO 5), 3235571 (SEQUENCE ID NO 6), 2680592 (SEQUENCE ID
35 NO 7), 608819 (SEQUENCE ID NO 8), 1629002 (SEQUENCE ID NO 9), 1626435 (SEQUENCE ID NO 10), 1582693 (SEQUENCE ID NO 11), 1281379 (SEQUENCE

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ID NO 12), 1628509 (SEQUENCE ID NO 13), 2060739 (SEQUENCE ID NO 14), 1624934 (SEQUENCE ID NO 15), 1800229 (SEQUENCE ID NO 16), 990829 (SEQUENCE ID NO 17), 1737775IH (SEQUENCE ID NO 18), and 608819IH (SEQUENCE ID NO 19), which form overlapping regions of the CS194 gene, and the resultant consensus nucleotide sequence (SEQUENCE ID NO 20) of these clones in a graphic display. Following this, a three-frame translation was performed on the consensus sequence (SEQUENCE ID NO 20). The first forward frame was found to have an open reading frame encoding a 914 residue amino acid sequence which is presented as SEQUENCE ID NO 41. The 914 residue polypeptide sequence depicted in SEQUENCE ID NO 41 was compared with published sequences using software and techniques known to those skilled in the art. The polypeptide sequence of a bovine epithelial chloride channel protein was found to be partially homologous to the CS194 polypeptide of SEQUENCE ID NO 41. This bovine chloride channel protein is described by S. Cunningham et al. *J. Biol. Chem.* 270:31016-31026 (1995).

Figure 1 also shows a T/C polymorphism at position 2460 in the consensus nucleotide sequence (SEQUENCE ID NO 20). As noted in the LIFESEQ™ database, the ratio of T's to C's at position 2460 was 9:2. However, this nucleotide polymorphism does not change the amino acid which is threonine in both cases (ACT vs. ACC).

Example 2: Sequencing of CS194 EST-Specific Clones

The full-length DNA sequences of clone 1737775, which comprises the 5'-most EST of the CS194 gene contig, and clone 608819 (also of the CS194 gene contig) were determined using dideoxy termination sequencing with dye terminators following known methods [F. Sanger et al., *PNAS U.S.A.* 74:5463 (1977)]. These full-length sequences are referred to herein as clones 1737775IH (SEQUENCE ID NO 18) and 608819IH (SEQUENCE ID NO 19), respectively.

Because the pINCY vector (available from Incyte Pharmaceuticals, Inc., Palo Alto, CA) contains universal priming sites just adjacent to the 3' and 5' ligation junctions of the inserts, approximately 300 bases of the insert were sequenced in both directions using two universal primers (SEQUENCE ID NO 23 and SEQUENCE ID NO 24, available from New England Biolabs, Beverly, MA, and Applied Biosystems Inc, Foster City, CA). The sequencing reactions were run on a polyacrylamide denaturing gel, and the sequences were determined by an Applied Biosystems 377 Sequencer (available from Applied Biosystems, Foster City, CA). Additional sequencing primers (SEQUENCE ID NOS 25-34 and SEQUENCE ID NOS 35-38)

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were designed from sequence information determined by the initial sequencing reactions near the 3'-ends of the two DNA strands of clones 608819 and 1737775, respectively. These primers then were used to determine the remaining DNA sequence of the cloned insert from each DNA strand, as previously described.

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Example 3: Nucleic Acid

A. RNA Extraction from Tissue. Total RNA was isolated from GI tract tissues and from non-GI tract tissues. Various methods were utilized, including but not limited to the lithium chloride/urea technique, known in the art and described by Kato et al. (J. Virol. 61:2182-2191, 1987), and TRIzol™ (Gibco-BRL, Grand Island, NY).

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Briefly, tissue was placed in a sterile conical tube on ice and 10-15 volumes of 3 M LiCl, 6 M urea, 5 mM EDTA, 0.1 M β-mercaptoethanol, 50 mM Tris-HCl (pH 7.5) were added. The tissue was homogenized with a Polytron® homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 30-50 sec on ice. The solution was transferred to a 15 ml plastic centrifuge tube and placed overnight at -20°C. The tube was centrifuged for 90 min at 9,000 x g at 0-4°C and the supernatant was immediately decanted. Ten ml of 3 M LiCl were added and the tube was vortexed for 5 sec. The tube was centrifuged for 45 min at 11,000 x g at 0-4°C. The decanting, resuspension in LiCl, and centrifugation was repeated and the final pellet was air dried and suspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 7.5). Twenty microliters (20 μl) of Proteinase K (20 mg/ml) were added, and the solution was incubated for 30 min at 37°C with occasional mixing. One-tenth volume (0.22-0.25 ml) of 3 M NaCl was added and the solution was vortexed before transfer into another tube containing 2 ml of phenol/chloroform/isoamyl alcohol (PCI). The tube was vortexed for 1-3 sec and centrifuged for 20 min at 3,000 x g at 10°C. The PCI extraction was repeated and followed by two similar extractions with chloroform/isoamyl alcohol (CI). The final aqueous solution was transferred to a prechilled 15 ml Corex glass tube containing 6 ml of absolute ethanol, the tube was covered with parafilm, and placed at -20°C overnight. The tube was centrifuged for 30 min at 10,000 x g at 0-4°C and the ethanol supernatant was decanted immediately. The RNA pellet was washed four times with 10 ml of 75% ice-cold ethanol and the final pellet was air dried for 15 min at room temperature. The RNA was suspended in 0.5 ml of 10 mM TE (pH 7.6, 1 mM EDTA) and its concentration was determined spectrophotometrically. RNA samples were aliquoted and stored at -70°C as ethanol precipitates.

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The quality of the RNA was determined by agarose gel electrophoresis (see Example 5, Northern Blot Analysis) and staining with 0.5 μg/ml ethidium bromide for

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one hour. RNA samples that did not contain intact rRNAs were excluded from the study.

Alternatively, for RT-PCR analysis, 1 ml of Ultraspec RNA reagent was added to 120 mg of pulverized tissue in a 2.0 ml polypropylene microfuge tube, homogenized with a Polytron® homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 50 sec and placed on ice for 5 min. Then, 0.2 ml of chloroform was added to each sample, followed by vortexing for 15 sec. The sample was placed on ice for another 5 min, followed by centrifugation at 12,000 x g for 15 min at 4°C. The upper layer was collected and transferred to another RNase-free 2.0 ml microfuge tube. An equal volume of isopropanol was added to each sample, and the solution was placed on ice for 10 min. The sample was centrifuged at 12,000 x g for 10 min at 4°C, and the supernatant was discarded. The remaining pellet was washed twice with cold 75% ethanol, resuspended by vortexing, and the resuspended material was then pelleted by centrifugation at 7500 x g for 5 min at 4°C. Finally, the RNA pellet was dried in a Speedvac (Savant, Farmingdale, NY) for 5 min and reconstituted in RNase-free water.

B. RNA Extraction from Blood Mononuclear Cells. Mononuclear cells are isolated from blood samples from patients by centrifugation using Ficoll-Hypaque as follows. A 10 ml volume of whole blood is mixed with an equal volume of RPMI Medium (Gibco-BRL, Grand Island, NY). This mixture is then underlayered with 10 ml of Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and centrifuged for 30 minutes at 200 x g. The buffy coat containing the mononuclear cells is removed, diluted to 50 ml with Dulbecco's PBS (Gibco-BRL, Grand Island, NY) and the mixture centrifuged for 10 minutes at 200 x g. After two washes, the resulting pellet is resuspended in Dulbecco's PBS to a final volume of 1 ml.

RNA is prepared from the isolated mononuclear cells as described by N. Kato et al., J. Virology 61: 2182-2191 (1987). Briefly, the pelleted mononuclear cells are brought to a final volume of 1 ml and then are resuspended in 250 µL of PBS and mixed with 2.5 ml of 3 M LiCl, 6 M urea, 5 mM EDTA, 0.1 M 2-mercaptoethanol, and 50 mM Tris-HCl (pH 7.5). The resulting mixture is homogenized and incubated at -20°C overnight. The homogenate is centrifuged at 8,000 RPM in a Beckman J2-21M rotor for 90 minutes at 0-4°C. The pellet is resuspended in 10 ml of 3 M LiCl by vortexing and then centrifuged at 10,000 RPM in a Beckman J2-21M rotor centrifuge for 45 minutes at 0-4°C. The resuspending and pelleting steps then are repeated. The pellet is resuspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 7.5) and 400 µg Proteinase K with vortexing and then it is incubated at 37°C for 30 minutes with shaking. One tenth volume of 3 M NaCl then is added and the mixture is vortexed.

Proteins are removed by two cycles of extraction with phenol/ chloroform/ isoamyl alcohol (PCI) followed by one extraction with chloroform/ isoamyl alcohol (CI). RNA is precipitated by the addition of 6 ml of absolute ethanol followed by overnight incubation at -20°C. After the precipitated RNA is collected by centrifugation, the pellet is washed 4 times in 75% ethanol. The pelleted RNA is then dissolved in solution containing 1 mM EDTA and 10 mM Tris-HCl (pH 7.5).

Non-GI tract tissues are used as negative controls. The mRNA can be further purified from total RNA by using commercially available kits such as oligo dT cellulose spin columns (RediCol™ from Pharmacia, Uppsala, Sweden) for the isolation of polyadenylated RNA. Total RNA or mRNA can be dissolved in lysis buffer (5 M guanidine thiocyanate and 0.1 M EDTA, pH 7.0) for analysis in the ribonuclease protection assay.

C. RNA Extraction from polysomes. Tissue is minced in saline at 4°C and mixed with 2.5 volumes of 0.8 M sucrose in a TK₁₅₀M (150 mM KCl, 5 mM MgCl₂, 50 mM Tris-HCl, pH 7.4) solution containing 6 mM 2-mercaptoethanol. The tissue is homogenized in a Teflon-glass Potter homogenizer with five strokes at 100-200 rpm followed by six strokes in a Dounce homogenizer, as described by B. Mechler, Methods in Enzymology 152:241-248 (1987). The homogenate then is centrifuged at 12,000 x g for 15 min at 4°C to sediment the nuclei. The polysomes are isolated by mixing 2 ml of the supernatant with 6 ml of 2.5 M sucrose in TK₁₅₀M and layering this mixture over 4 ml of 2.5 M sucrose in TK₁₅₀M in a 38 ml polyallomer tube. Two additional sucrose TK₁₅₀M solutions are successively layered onto the extract fraction; a first layer of 13 ml 2.05 M sucrose followed by a second layer of 6 ml of 1.3 M sucrose. The polysomes are isolated by centrifuging the gradient at 90,000 x g for 5 hr at 4°C. The fraction then is taken from the 1.3 M sucrose/2.05 M sucrose interface with a siliconized pasteur pipette and diluted in an equal volume of TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). An equal volume of 90°C SDS buffer (1% SDS, 200 mM NaCl, 20 mM Tris-HCl, pH 7.4) is added and the solution is incubated in a boiling water bath for 2 min. Proteins next are digested with a Proteinase-K digestion (50 mg/ml) for 15 min at 37°C. The mRNA is purified with 3 equal volumes of phenol-chloroform extractions followed by precipitation with 0.1 volume of 2 M sodium acetate (pH 5.2) and 2 volumes of 100% ethanol at -20°C overnight. The precipitated RNA is recovered by centrifugation at 12,000 x g for 10 min at 4°C. The RNA is dried and resuspended in TE (pH 7.4) or distilled water. The resuspended RNA then can be used in a slot blot or dot blot hybridization assay to check for the presence of CS194 mRNA (see Example 6).

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The quality of nucleic acid and proteins is dependent on the method of preparation used. Each sample may require a different preparation technique to maximize isolation efficiency of the target molecule. These preparation techniques are within the skill of the ordinary artisan.

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Example 4: Ribonuclease Protection Assay

A. Synthesis of Labeled Complementary RNA (cRNA) Hybridization Probe and Unlabeled Sense Strand. Labeled antisense and unlabeled sense riboprobes are transcribed from the CS194 gene cDNA sequence which contains a 5' RNA polymerase promoter such as SP6 or T7. The sequence may be from a vector containing the appropriate CS194 cDNA insert, or from a PCR-generated product of the insert using PCR primers which incorporate a 5' RNA polymerase promoter sequence. For example, the described plasmid, clones 1737775 and 608819 or another comparable clone, containing the CS194 gene cDNA sequence, flanked by opposed SP6 and T7 polymerase promoters, is purified using Qiagen Plasmid Purification Kit (Qiagen, Chatsworth, CA). Then 10 µg of the plasmid are linearized by cutting with 10 U DdeI restriction enzyme for 1 hr at 37°C. The linearized plasmid is purified using QIAprep kits (Qiagen, Chatsworth, CA) and used for the synthesis of antisense transcript from the appropriate SP6 or T7 promoter using the Riboprobe® in vitro Transcription System (Promega Corporation, Madison, WI); as described by the supplier's instructions, incorporating either 6.3 µM (alpha-³²P) UTP (Amersham Life Sciences, Inc. Arlington Heights, IL) or 100-500 µM biotinylated UTP as a label. To generate the sense strand, 10 µg of the purified plasmid are cut with restriction enzymes 10U XbaI and 10 U NotI, and transcribed as above from the appropriate SP6 or T7 promoter. Both sense and antisense strands are isolated by spin column chromatography. Unlabeled sense strand is quantitated by UV absorption at 260 nm.

B. Hybridization of Labeled Probe to Target. Frozen tissue is pulverized to powder under liquid nitrogen and 100-500 mg are dissolved in 1 ml of lysis buffer, available as a component of the Direct Protect™ Lysate RNase Protection kit (Ambion, Inc., Austin, TX). Further dissolution can be achieved using a tissue homogenizer. In addition, a dilution series of a known amount of sense strand in mouse liver lysate is made for use as a positive control. Finally, 45 µl of solubilized tissue or diluted sense strand are mixed directly with either (1) 1 x 10⁵ cpm of radioactively labeled probe, or (2) 250 pg of non-isotopically labeled probe in 5 µl of lysis buffer. Hybridization is allowed to proceed overnight at 37°C. See, T. Kaabache et al., Anal. Biochem. 232:225-230 (1995).

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C. RNase Digestion. RNA that is not hybridized to probe is removed from the reaction as per the Direct Protect™ protocol using a solution of RNase A and RNase T1 for 30 min at 37°C, followed by removal of RNase by Proteinase-K digestion in the presence of sodium sarcosyl. Hybridized fragments protected from digestion are then precipitated by the addition of an equal volume of isopropanol and placed at -70°C for 3 hr. The precipitates are collected by centrifugation at 12,000 x g for 20 min.

D. Fragment Analysis. The precipitates are dissolved in denaturing gel loading dye (80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml xylene cyanol, 1 mg/ml bromophenol blue), heat denatured, and electrophoresed in 6% polyacrylamide TBE, 8 M urea denaturing gels. The gels are imaged and analyzed using the STORM™ storage phosphor autoradiography system (Molecular Dynamics, Sunnyvale, CA). Quantitation of protected fragment bands, expressed in femtograms (fg), is achieved by comparing the peak areas obtained from the test samples to those from the known dilutions of the positive control sense strand (see Section B, supra). The results are expressed in molecules of CS194 RNA/cell and as a image rating score. In cases where non-isotopic labels are used, hybrids are transferred from the gels to membranes (nylon or nitrocellulose) by blotting and then analyzed using detection systems that employ streptavidin alkaline phosphatase conjugates and chemiluminescence or chemifluorescence reagents.

Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, is indicative of the presence of CS194 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Example 5: Northern Blotting

The Northern blot technique was used to identify a specific size RNA species in a complex population of RNA using agarose gel electrophoresis and nucleic acid hybridization. Briefly, 5-10 µg of total RNA (see Example 3, Nucleic Acid Preparation) were incubated in 15 µl of a solution containing 40 mM morpholinopropanesulfonic acid (MOPS) (pH 7.0), 10 mM sodium acetate, 1 mM EDTA, 2.2 M formaldehyde, 50% v/v formamide for 15 min at 65°C. The denatured RNA was mixed with 2 µl of loading buffer (50% glycerol, 1 mM EDTA, 0.4% bromophenol blue, 0.4% xylene cyanol) and loaded into a denaturing 1.0% agarose gel containing 40 mM MOPS (pH 7.0), 10 mM sodium acetate, 1 mM EDTA and 2.2 M formaldehyde. The gel was electrophoresed at 60 V for 1.5 hr, stained with 0.5 µg/ml ethidium bromide for one hour and rinsed in RNase free water for 30-45 min. RNA

was transferred from the gel onto nylon membranes (Brightstar-Plus, Ambion, Inc., Austin, TX) for 1.5 hours using the downward alkaline capillary transfer method (Chomczynski, Anal. Biochem. 201:134-139, 1992). The filter was rinsed with 1X SSC and RNA was crosslinked to the filter using a Stratalinker (Stratagene, Inc., La Jolla, CA) on the autocrosslinking mode and dried for 15 min. The membrane was then placed into a hybridization tube containing 20 ml of preheated prehybridization solution (5X SSC, 50% formamide, 5X Denhardt's solution, 100 µg/ml denatured salmon sperm DNA) and incubated in a 42°C hybridization oven for at least 3 hr. While the blot was prehybridizing, a ³²P-labeled random-primed probe was generated using the CS194 insert according to the manufacturer's instructions (Gibco-BRL, Grand Island, NY). Half of the probe was boiled for 10 min, quick chilled on ice and added to the hybridization tube. Hybridization was carried out at 42°C for at least 12 hr. The hybridization solution was discarded and the filter was washed twice in 30 ml of 3X SSC, 0.1% SDS at 42°C for 15 min, followed by two washes in 30 ml of 0.3X SSC, 0.1% SDS at 60°C for 15 min. each. The filter was wrapped in Saran Wrap and exposed to Kodak XAR-Omat film for 8-120 hr and the film was developed for analysis.

The CS194 probe detected an approximately 0.75 Kb RNA in 2 of 6 normal colon specimens and in all 6 colon cancer specimens.

Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, is indicative of the presence of CS194 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Example 6: Dot Blot/Slot Blot

Dot and slot blot assays are quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid. To perform such assays, up to 50 µg of RNA are mixed in 50 µl of 50% formamide, 7% formaldehyde, 1X SSC, incubated 15 min at 68°C, and then cooled on ice. Then, 100 µl of 20X SSC are added to the RNA mixture and loaded under vacuum onto a manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described in Example 4, supra. Detection of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, is an indication of

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the presence of CS194, suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Other methods and buffers which can be utilized in the methods described in Examples 5 and 6, but not specifically detailed herein, are known in the art and are
5 described in J. Sambrook et al, supra which is incorporated herein by reference.

Example 7: In Situ Hybridization

This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

10 Tissues are prepared with cross-linking fixative agents such as paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer et al., Methods in Cell Biol. 35:37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 min. The solution is changed with fresh glutaraldehyde solution (1%
15 glutaraldehyde in 50 mM sodium phosphate, pH 7.5) for a further 30 min fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

The fixed tissues then are embedded in paraffin as follows. The tissue is dehydrated through a series of increasing ethanol concentrations for 15 min each: 50%
20 (twice), 70% (twice), 85%, 90% and then 100% (twice). Next, the tissue is soaked in two changes of xylene for 20 min each at room temperature. The tissue is then soaked in two changes of a 1:1 mixture of xylene and paraffin for 20 min each at 60°C; and then in three final changes of paraffin for 15 min each.

Next, the tissue is cut in 5 µm sections using a standard microtome and placed
25 on a slide previously treated with a tissue adhesive such as 3-aminopropyltriethoxysilane.

Paraffin is removed from the tissue by two 10 min xylene soaks and rehydrated in a series of decreasing ethanol concentrations: 99% (twice), 95%, 85%, 70%, 50%, 30%, and then in distilled water (twice). The sections are pre-treated with 0.2 M HCl
30 for 10 min and permeabilized with 2 µg/ml Proteinase-K at 37°C for 15 min.

Labeled Riboprobes transcribed from the CS194 gene plasmid (see Example 4) are hybridized to the prepared tissue sections and incubated overnight at 56°C in 3X standard saline extract and 50% formamide. Excess probe is removed by washing in 2X standard saline citrate and 50% formamide followed by digestion with 100 µg/ml
35 RNase A at 37°C for 30 min. Fluorescence probe is visualized by illumination with

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ultraviolet (UV) light under a microscope. Fluorescence in the cytoplasm is indicative of CS194 mRNA. Alternatively, the sections can be visualized by autoradiography.

Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, is indicative of the presence of CS194 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Example 8: Reverse Transcription PCR

A. One Step RT-PCR Assay. Target-specific primers were designed to detect the above-described target sequences by reverse transcription PCR using methods known in the art. One step RT-PCR is a sequential procedure that performs both RT and PCR in a single reaction mixture. The procedure is performed in a 200 µl reaction mixture containing 50 mM (N,N-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN₃, 8% w/v glycerol, 150 µM each of dNTP, 0.25 µM each primer, 5U rTth polymerase, 3.25 mM Mn(OAc)₂ and 5 µl of target RNA (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of Mn(OAc)₂, the Mn(OAc)₂ should be added just before target addition. Optimal conditions for cDNA synthesis and thermal cycling readily can be determined by those skilled in the art. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can readily be determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis at 60°-70°C for 15-45 min and 30-45 amplification cycles at 94°C, 1 min; 55°-70°C, 1 min; 72°C, 2 min. One step RT-PCR also may be performed by using a dual enzyme procedure with Taq polymerase and a reverse transcriptase enzyme, such as MMLV or AMV RT enzymes.

B. Traditional RT-PCR. A traditional two-step RT-PCR reaction is performed, as described by K.Q. Hu et al., *Virology* 181:721-726 (1991). Briefly, 0.5 µg of extracted mRNA (see Example 3) is reverse transcribed in a 20 µl reaction mixture containing 1X PCR II buffer (Perkin-Elmer), 5 mM MgCl₂, 1 mM dNTP, 20 U RNasin, 2.5 µM random hexamers, and 50 U MMLV (Moloney murine leukemia virus) reverse transcriptase (RT). Reverse transcription is performed at room temperature for 10 min, 42°C for 60 min in a PE-480 thermal cycler, followed by further incubation at 95°C for 5 min to inactivate the RT. PCR is performed using 2 µl of the cDNA reaction in a final PCR reaction volume of 50 µl containing 10 mM Tris-

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HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTP, 0.4 μM of each sense and antisense primer, SEQUENCE ID NO 39 (5'-CCACCTCACTCCTGATTCA TTGC-3') and SEQUENCE ID NO 40 (5'-TGATTCAAGTCGATTGAGGCGGTT AC-3'), respectively, and 2.5 U of Taq polymerase. The reaction is incubated in an MJ Research Model PTC-200 as follows: Denaturation at 94°C for 2 min. followed by 35 cycles of amplification (94°C, 45 sec; 55°C, 45 sec; 72°C, 2 min); a final extension (72°C, 5 min); and a soak at 4°C.

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C. PCR Fragment Analysis. The correct products are verified by size determination using gel electrophoresis. After the gel is stained with ethidium bromide (0.5 μg/ml in TBE buffer) for 15 minutes and destained in water for 10 minutes, it is visualized by UV illumination.

Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, is indicative of the presence of CS194 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

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Example 9: OH-PCR

A. Probe selection and Labeling. Target-specific primers and probes are designed to detect the above-described target sequences by oligonucleotide hybridization PCR. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling oligonucleotides at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. For example, see N. T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' end to prevent participation in PCR and the formation of undesired extension products. For one step OH-PCR, the probe should have a T_M at least 15°C below the T_M of the primers. The primers and probes are utilized as specific binding members, with or without detectable labels, using standard phosphoramidite chemistry and/or post-synthetic labeling methods which are well-known to one skilled in the art.

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B. One Step Oligo Hybridization PCR. OH-PCR is performed on a 200 μl reaction containing 50 mM (N,N-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN₃, 8% w/v glycerol, 150 μM each of dNTP,

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0.25 μ M each primer, 3.75 nM probe, 5U rTth polymerase, 3.25 mM Mn(OAc)₂ and 5 μ l blood equivalents of target (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of Mn(OAc)₂, the Mn(OAc)₂ should be added just before target addition. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480.

- 5 Optimal conditions for cDNA synthesis and thermal cycling can be readily determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis (60°C, 30 min), 30-45 amplification cycles (94°C, 40 sec; 55-70°C, 60 sec), oligo-hybridization (97°C, 5 min; 15°C, 5 min; 15°C soak). The correct reaction product contains at least one of the strands of the PCR product and an internally
10 hybridized probe.

- C. OH-PCR Product Analysis. Amplified reaction products are detected on an LCx[®] analyzer system (available from Abbott Laboratories, Abbott Park, IL). Briefly, the correct reaction product is captured by an antibody labeled microparticle at a capturable site on either the PCR product strand or the hybridization probe, and the
15 complex is detected by binding of a detectable antibody conjugate to either a detectable site on the probe or the PCR strand. Only a complex containing a PCR strand hybridized with the internal probe is detectable. The detection of this complex then is indicative of the presence of CS194 mRNA, suggesting a diagnosis of a GI tract disease or condition, such as GI tract cancer.

- 20 Many other detection formats exist which can be used and/or modified by those skilled in the art to detect the presence of amplified or non-amplified CS194-derived nucleic acid sequences including, but not limited to, ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL); Q-beta replicase (Gene-Trak[™], Naperville, Illinois), branched chain reaction (Chiron, Emeryville, CA) and strand displacement assays
25 (Becton Dickinson, Research Triangle Park, NC).

Example 10: Synthetic Peptide Production

- Synthetic peptides were modeled and then prepared based upon the predicted amino acid sequence of the CS194 polypeptide consensus sequence (see Example 1).
30 In particular, a number of CS194 peptides modeled from SEQUENCE ID NO 41 were prepared, including the peptides of SEQUENCE ID NO 42, SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, SEQUENCE ID NO 46, SEQUENCE ID NO 47, SEQUENCE ID NO 48, and SEQUENCE ID NO 49. All peptides were synthesized on a Symphony Peptide Synthesizer (available from Rainin
35 Instrument Co, Emeryville, CA) using FMOC chemistry, standard cycles and in-situ HBTU activation. Cleavage and deprotection conditions were as follows: a volume of

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2.5 ml of cleavage reagent (77.5% v/v trifluoroacetic acid, 15% v/v ethanedithiol, 2.5% v/v water, 5% v/v thioanisole, 1-2% w/v phenol) were added to the resin, and agitated at room temperature for 2-4 hours. Then the filtrate was removed and the peptide was precipitated from the cleavage reagent with cold diethyl ether. Each peptide was filtered, purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient, and lyophilized. The product was confirmed by mass spectrometry (see Example 12).

The purified peptides were used to immunize animals (see Example 14).

Example 11a: Expression of Protein in a Cell Line Using Plasmid 577

A. Construction of a CS194 Expression Plasmid. Plasmid 577, described in

U.S. patent application Serial No. 08/478,073, filed June 7, 1995 and incorporated herein by reference, has been constructed for the expression of secreted antigens in a permanent cell line. This plasmid contains the following DNA segments: (a) a 2.3 Kb fragment of pBR322 containing bacterial beta-lactamase and origin of DNA replication; (b) a 1.8 Kb cassette directing expression of a neomycin resistance gene under control of HSV-1 thymidine kinase promoter and poly-A addition signals; (c) a 1.9 Kb cassette directing expression of a dihydrofolate reductase gene under the control of an SV-40 (Simian Virus 40) promoter and poly-A addition signals; (d) a 3.5 Kb cassette directing expression of a rabbit immunoglobulin heavy chain signal sequence fused to a modified hepatitis C virus (HCV) E2 protein under the control of the SV40 T-Ag promoter and transcription enhancer, the hepatitis B virus surface antigen (HBsAg) enhancer I followed by a fragment of Herpes Simplex Virus-1 (HSV-1) genome providing poly-A addition signals; and (e) a residual 0.7 Kb fragment of SV40 genome late region of no function in this plasmid. All of the segments of the vector were assembled by standard methods known to those skilled in the art of molecular biology.

Plasmids for the expression of secretable CS194 proteins are constructed by replacing the hepatitis C virus E2 protein coding sequence in plasmid 577 with that of a CS194 polynucleotide sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof, as follows. Digestion of plasmid 577 with XbaI releases the hepatitis C virus E2 gene fragment. The resulting plasmid backbone allows insertion of the CS194 cDNA insert downstream of the rabbit immunoglobulin heavy chain signal sequence which directs the expressed proteins into the secretory pathway of the cell. The CS194 cDNA fragment is generated by PCR using standard procedures. Encoded in the sense PCR primer sequence is an XbaI site, immediately followed by a 12 nucleotide sequence that encodes the amino acid sequence

Ser-Asn-Glu-Leu ("SNEL") to promote signal protease processing, efficient secretion and final product stability in culture fluids. Immediately following this 12 nucleotide sequence the primer contains nucleotides complementary to template sequences encoding amino acids of the CS194 gene. The antisense primer incorporates a sequence encoding the following eight amino acids just before the stop codons: Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQUENCE ID NO 50). Within this sequence is incorporated a recognition site to aid in analysis and purification of the CS194 protein product. A recognition site (termed "FLAG") that is recognized by a commercially available monoclonal antibody designated anti-FLAG M2 (Eastman Kodak, Co., New Haven, CT) can be utilized, as well as other comparable sequences and their corresponding antibodies. For example, PCR is performed using GeneAmp® reagents obtained from Perkin-Elmer-Cetus, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5 µM. PCR is performed on the CS194 plasmid template in a 100 µl reaction for 35 cycles (94°C, 30 seconds; 55°C, 30 seconds; and 72°C, 90 seconds) followed by an extension cycle of 72°C for 10 min.

B. Transfection of Dihydrofolate Reductase Deficient Chinese Hamster Ovary Cells. The plasmid described supra is transfected into CHO/dhfr- cells (DXB-111, Uriacio et al., PNAS 77:4451-4466 (1980)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL 9096. Transfection is carried out using the cationic liposome-mediated procedure described by P. L. Felgner et al., PNAS 84:7413-7417 (1987). Particularly, CHO/dhfr- cells are cultured in Ham's F-12 media supplemented with 10% fetal calf serum, L-glutamine (1 mM) and freshly seeded into a flask at a density of 5-8 x 10⁵ cells per flask. The cells are grown to a confluency of between 60 and 80% for transfection. Twenty micrograms (20µg) of plasmid DNA is added to 1.5 ml of Opti-MEM I medium and 100 µl of Lipofectin Reagent (Gibco-BRL; Grand Island, NY) are added to a second 1.5 ml portion of Opti-MEM I media. The two solutions are mixed and incubated at room temperature for 20 min. After the culture medium is removed from the cells, the cells are rinsed 3 times with 5 ml of Opti-MEM I medium. The Opti-MEM I-Lipofection-plasmid DNA solution then is overlaid onto the cells. The cells are incubated for 3 h at 37°C, after which time the Opti-MEM I-Lipofectin-DNA solution is replaced with culture medium for an additional 24 h prior to selection.

C. Selection and Amplification. One day after transfection, cells are passaged 1:3 and incubated with dhfr/G418 selection medium (hereafter, "F-12 minus medium G"). Selection medium is Ham's F-12 with L-glutamine and without hypoxanthine, thymidine and glycine (JRH Biosciences, Lenexa, Kansas) and 300 µg per ml G418

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(Gibco-BRL; Grand Island, NY). Media volume-to-surface area ratios of 5 ml per 25 cm² are maintained. After approximately two weeks, DHFR/G418 cells are expanded to allow passage and continuous maintenance in F-12 minus medium G.

Amplification of each of the transfected CS194 cDNA sequences is achieved by stepwise selection of DHFR⁺, G418⁺ cells with methotrexate (reviewed by R. Schimke, Cell 37:705-713 [1984]). Cells are incubated with F-12 minus medium G containing 150 nM methotrexate (MTX) (Sigma, St. Louis, MO) for approximately two weeks until resistant colonies appear. Further gene amplification is achieved by selection of 150 nM adapted cells with 5 μ M MTX.

D. Antigen Production. F-12 minus medium G supplemented with 5 μ M MTX is overlaid onto just confluent monolayers for 12 to 24 h at 37°C in 5% CO₂. The growth medium is removed and the cells are rinsed 3 times with Dulbecco's phosphate buffered saline (PBS) with calcium and magnesium (Gibco-BRL, Grand Island, NY) to remove the remaining media/serum which may be present. Cells then are incubated with VAS custom medium (VAS custom formulation with L-glutamine with HEPES without phenol red, available from JRH Bioscience; Lenexa, KS, product number 52-08678P), for 1 h at 37°C in 5% CO₂. Cells then are overlaid with VAS for production at 5 ml per T flask. Medium is removed after seven days of incubation, retained, and then frozen to await purification with harvests 2, 3 and 4. The monolayers are overlaid with VAS for 3 more seven day harvests.

E. Analysis of GI tract Tissue Gene CS194 Antigen Expression. Aliquots of VAS supernatants from the cells expressing the CS194 protein construct are analyzed, either by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using standard methods and reagents known in the art (Laemmli discontinuous gels), or by mass spectrometry.

F. Purification. Purification of the CS194 protein containing the FLAG sequence is performed by immunoaffinity chromatography using an affinity matrix comprising anti-FLAG M2 monoclonal antibody covalently attached to agarose by hydrazide linkage (Eastman Kodak Co., New Haven, CT). Prior to affinity purification, protein in pooled VAS medium harvests from roller bottles is exchanged into 50 mM Tris-HCl (pH 7.5), 150 mM NaCl buffer using a Sephadex G-25 (Pharmacia Biotech Inc., Uppsala, Sweden) column. Protein in this buffer is applied to the anti-FLAG M2 antibody affinity column. Non-binding protein is eluted by washing the column with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl buffer. Bound protein is eluted using an excess of FLAG peptide in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl.

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The excess FLAG peptide can be removed from the purified CS194 protein by gel electrophoresis or HPLC.

Although plasmid 577 is utilized in this example, it is known to those skilled in the art that other comparable expression systems, such as CMV, can be utilized herein with appropriate modifications in reagent and/or techniques and are within the skill of the ordinary artisan.

The largest cloned insert containing the coding region of the CS194 gene is then sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible sequences which aid in protein expression and detection, or (ii) a bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in EPO 0196056, published October 1, 1986, which is incorporated herein by reference and those containing fusion sequences of CKS are described in EPO Publication No. 0331961, published September 13, 1989, which publication is also incorporated herein by reference. This so-purified protein can be used in a variety of techniques, including, but not limited to animal immunization studies, solid phase immunoassays, etc.

Example 11b: Expression of Protein in a Cell Line Using pcDNA3.1/Myc-His

A. Construction of a CS194 Expression Plasmid. Plasmid pcDNA3.1/Myc-His (Cat. # V855-20, Invitrogen, Carlsbad, CA) has been constructed, in the past, for the expression of secreted antigens by most mammalian cell lines. Expressed protein inserts are fused to a myc-his peptide tag. The myc-his tag is a 21 residue amino acid sequence having the following sequence: Glu-Gln-Lys-Leu-Ile-Ser-Glu- Glu-Asp-Leu-Asn-Met-His-Thr-Glu-His-His-His-His-His (SEQUENCE ID NO 51) and comprises a myc epitope and a polyhistidine sequence which are useful for the purification of an expressed fusion protein using either anti-myc or anti-his affinity columns, or metalloprotein binding columns.

Plasmids for the expression of secretable CS194 proteins are constructed by inserting an CS194 polynucleotide sequence selected from the group consisting of SEQUENCE ID NOS 1-20, and fragments or complements thereof. Prior to construction of an CS194 expression plasmid, the CS194 cDNA sequence is first cloned into a pCR®-Blunt vector as follows.

The CS194 cDNA fragment is generated by PCR using standard procedures. For example, PCR is performed using Stratagene® reagents obtained from Stratagene,

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La Jolla, CA, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5 μ M. PCR using 5 U of pfu polymerase (Stratagene) is performed on the CS194 plasmid template (see Example 2) in a 50 μ l reaction for 30 cycles (94°C, 1 min; 65°C, 1.5 min; 72°C, 3 min) followed by an extension cycle at 72°C for 8 min.

- 5 The sense PCR primer sequence comprises nucleotides which are either complementary to the pINCY vector directly upstream of the CS194 gene insert or which incorporate a 5' EcoRI restriction site, an adjacent downstream protein translation consensus initiator, and a 3' nucleic acid sequence which is the same sense as the 5'-most end of the CS194 cDNA insert. The antisense primer incorporates a 5' NotI restriction sequence and a
- 10 sequence complementary to the 3' end of the CS194 cDNA insert just upstream of the 3'-most, in-frame stop codon. Five microliters (5 μ l) of the resulting blunt-ended PCR product are ligated into 25 ng of linearized pCR[®]-Blunt vector (Invitrogen, Carlsbad, CA) interrupting the lethal ccdB gene of the vector. The resulting ligated vector is transfected into TOP10 *E. coli* (Invitrogen, Carlsbad, CA) using a One Shot[™]
- 15 transformation kit (Invitrogen, Carlsbad, CA) following the supplier's directions. The transfected cells are grown on LB-Kan (50 μ g/ml kanamycin) selection plates at 37°C. Only cells containing a plasmid with an interrupted ccdB gene will grow after transfection (Grant, S.G.N., *PNAS USA* 87:4645-4649 (1990)). Transfected colonies are picked and grown up in 3 ml of LB-Kan broth at 37°C. Plasmid DNA is isolated
- 20 using a QIAprep[®] (Qiagen Inc., Santa Clarita, CA) procedure, as directed by the supplier's instructions. The DNA is cut with EcoRI or SnaBI, and NotI restriction enzymes to release the CS194 insert fragment. The fragment is run on 1% Seakem[®] LE agarose/0.5 μ g/ml ethidium bromide/TE gel, visualized by UV irradiation, excised and purified using QIAquick[™] (Qiagen Inc., Santa Clarita, CA) procedures, as directed by
- 25 the supplier's instructions.

- 30 The pcDNA3.1/Myc-His plasmid DNA is linearized by digestion with EcoRI or SnaBI, and NotI in the polylinker region of the plasmid DNA. The resulting plasmid DNA backbone allows insertion of the CS194 purified cDNA fragment, supra, downstream of a CMV promoter which directs expression of the proteins in mammalian cells. The ligated plasmid is transfected into DH5 alpha[™] cells (GibcoBRL, Gaithersburg, MD) as directed by the supplier's instructions. Briefly, 10 ng of pcDNA3.1/Myc-His containing an CS194 insert is added to 50 μ l of competent DH5 alpha cells, and the contents are mixed gently. The mixture is incubated on ice for 30 min, heat shocked for 20 sec at 37°C, and placed on ice for an additional 2 min. Upon
- 35 addition of 0.95 ml of LB medium, the mixture is incubated for 1 h at 37°C while shaking at 225 rpm. The transfected cells are then plated onto 100 mm LB/Amp (50

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µg/ml ampicillin) plates and grown at 37°C. Colonies are picked and grown in 3 ml of LB/Amp broth. Plasmid DNA is purified using a QIAprep® kit. Presence of the insert is confirmed using techniques known to those skilled in the art including, but not limited to, restriction digestion and gel analysis. See, e.g., J. Sambrook et al., supra.

5 B. Transfection of Human Embryonic Kidney 293 Cells. The CS194 expression plasmid described supra is purified from the DH5 alpha cells using a QIAfilter™ Maxi kit (Qiagen, Chatsworth, CA), and then transfected into HEK293 cells (F.L. Graham et al., J. Gen. Vir. 36:59-72 (1977)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL
10 1573. Transfection is carried out using the cationic lipofectamine-mediated procedure described by P. Hawley-Nelson et al., Focus 15:73 (1993). Particularly, HEK293 cells are cultured in 10 ml DMEM media supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM) and freshly seeded into 100 mm culture plates at a density of 9×10^6 cells per plate. The cells are grown at 37°C to a confluency of between 70%
15 and 80% for transfection. Eight micrograms (8 µg) of plasmid DNA is added to 800 µl of Opti-MEM I® medium (Gibco-BRL, Grand Island, NY), and 48-96 µl of Lipofectamine™ Reagent (Gibco-BRL, Grand Island, NY) is added to a second 800 µl portion of Opti-MEM I® media. The two solutions are mixed and incubated at room temperature for 15-30 min. After the culture medium is removed from the cells, the
20 cells are washed once with 10 ml of serum-free DMEM. The Opti-MEM I®-Lipofectamine-plasmid DNA solution is diluted in 6.4 ml of serum-free DMEM and then overlaid onto the cells. The cells are incubated for 5 h at 37°C, after which time, an additional 8 ml of DMEM with 20% FBS is added. After 18-24 h, the old medium is aspirated, and the cells are overlaid with 5 ml of fresh DMEM with 10% FBS.
25 Supernatants and cell extracts are analyzed for CS194 gene activity 72 h after transfection.

C. Analysis of GI Tract Tissue Gene CS194 Antigen Expression. The culture supernatant , supra, is transferred to cryotubes and stored on ice. HEK293 cells are harvested by washing twice with 10 ml cold Dulbecco's PBS and lysing by addition of
30 1.5 ml of CAT lysis buffer (Boehringer Mannheim, Indianapolis, IN), followed by incubation for 30 min at room temperature. Lysate is transferred to 1.7 ml polypropylene microfuge tubes and centrifuged at 1000 x g for 10 min. The supernatant is transferred to new cryotubes and stored on ice. Aliquots of cell supernatants and the lysate of the cells expressing the CS194 protein construct are
35 analyzed for the presence of CS194 recombinant protein. The aliquots can be analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using standard methods

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and reagents known in the art. See, e.g., J. Sambrook et al., supra. The gels can then be blotted onto a solid medium such as nitrocellulose, nytran, or the like, and the CS194 protein band can be visualized using western blotting techniques with anti-myc epitope or anti-histidine monoclonal antibodies (Invitrogen, Carlsbad, CA) or CS194 polyclonal serum (see Example 14). Alternatively, the expressed CS194 recombinant protein can be analyzed by mass spectrometry (see Example 12).

D. Purification. Purification of the CS194 recombinant protein containing the myc-his sequence is performed using the Xpress[®] affinity chromatography system (Invitrogen, Carlsbad, CA) containing a nickel-charged agarose resin which specifically binds polyhistidine residues. Supernatants from 10 x 100 mm plates, prepared as described supra, are pooled and passed over the nickel-charged column. Non-binding protein is eluted by washing the column with 50 mM Tris-HCl (pH 7.5)/150 mM NaCl buffer, leaving only the myc-his fusion proteins. Bound CS194 recombinant protein then is eluted from the column using either an excess of imidazole or histidine, or a low pH buffer. Alternatively, the recombinant protein can also be purified by binding at the myc-his sequence to an affinity column consisting of either anti-myc or anti-histidine monoclonal antibodies conjugated through a hydrazide or other linkage to an agarose resin and eluting with an excess of myc peptide or histidine, respectively.

The purified recombinant protein can then be covalently cross-linked to a solid phase, such as N-hydroxysuccinimide-activated sepharose columns (Pharmacia Biotech, Piscataway, NJ), as directed by supplier's instructions. These columns containing covalently linked CS194 recombinant protein, can then be used to purify anti-CS194 antibodies from rabbit or mouse sera (see Examples 13 and 14).

E. Coating Microtiter Plates with CS194 Expressed Proteins. Supernatant from a 100 mm plate, as described supra, is diluted in an appropriate volume of PBS. 100 µl of the resulting mixture is placed into each well of a Reacti-Bind[™] metal chelate microtiter plate (Pierce, Rockford, IL), incubated at room temperature while shaking, and followed by three washes with 200 µl each of PBS with 0.05% Tween[®] 20. The prepared microtiter plate can then be used to screen polyclonal antisera for the presence of CS194 antibodies (see Example 17).

Although pcDNA3.1/Myc-His is utilized in this example, it is known to those skilled in the art that other comparable expression systems can be utilized herein with appropriate modifications in reagent and/or techniques and are within the skill of one of ordinary skill in the art. The largest cloned insert containing the coding region of the CS194 gene is sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible

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sequences which aid in protein expression and detection, or (ii) a bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European patent application No. EP 0 196 056, published October 1, 1986, which is incorporated herein by reference, and vectors containing fusion sequences of CKS are described in European patent application No. EP 0 331 961, published September 13, 1989, which publication is also incorporated herein by reference. The purified protein can be used in a variety of techniques, including but not limited to, animal immunization studies, solid phase immunoassays, etc.

Example 12: Chemical Analysis of GI tract Tissue Proteins

A. Analysis of Tryptic Peptide Fragments Using MS. Sera from patients with GI tract disease, such as GI tract cancer, sera from patients with no GI tract disease, extracts of GI tract tissues or cells from patients with GI tract disease, such as GI tract cancer, extracts of GI tract tissues or cells from patients with no GI tract disease, and extracts of tissues or cells from other non-diseased or diseased organs of patients, are run on a polyacrylamide gel using standard procedures and stained with Coomassie Blue. Sections of the gel suspected of containing the unknown polypeptide are excised and subjected to an in-gel reduction, acetamidation and tryptic digestion. P. Jenö et al, Anal. Bio. 224:451-455 (1995) and J. Rosenfeld et al, Anal. Bio. 203:173-179 (1992). The gel sections are washed with 100 mM NH_4HCO_3 and acetonitrile. The shrunken gel pieces are swollen in digestion buffer (50 mM NH_4HCO_3 , 5 mM CaCl_2 and 12.5 $\mu\text{g/ml}$ trypsin) at 4°C for 45 min. The supernatant is aspirated and replaced with 5 to 10 μl of digestion buffer without trypsin and allowed to incubate overnight at 37°C. Peptides are extracted with 3 changes of 5% formic acid and acetonitrile and evaporated to dryness. The peptides are adsorbed to approximately 0.1 μl of POROS R2 sorbent (Perseptive Biosystems, Framingham, Massachusetts) trapped in the tip of a drawn gas chromatography capillary tube by dissolving them in 10 μl of 5% formic acid and passing it through the capillary. The adsorbed peptides are washed with water and eluted with 5% formic acid in 60% methanol. The eluant is passed directly into the spraying capillary of an API III mass spectrometer (Perkin-Elmer Sciex, Thornhill, Ontario, Canada) for analysis by nano-electrospray mass spectrometry. M. Wilm et al., Int. J. Mass Spectrom. Ion Process 136:167-180 (1994) and M. Wilm et al., Anal. Chem. 66:1-8 (1994). The masses of the tryptic peptides are determined from the mass

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spectrum obtained off the first quadrupole. Masses corresponding to predicted peptides can be further analyzed in MS/MS mode to give the amino acid sequence of the peptide.

5 B. Peptide Fragment Analysis Using LC/MS. The presence of polypeptides predicted from mRNA sequences found in hyperplastic disease tissues also can be confirmed using liquid chromatography/and em mass spectrometry (LC/MS/MS). D. Hess et al., METHODS, A Companion to Methods in Enzymology 6:227-238 (1994). The serum specimen or tumor extract from the patient is denatured with SDS and reduced with dithiothreitol (1.5 mg/ml) for 30 min at 90°C followed by alkylation with iodoacetamide (4 mg/ml) for 15 min at 25°C. Following acrylamide electrophoresis, the
10 polypeptides are electroblotted to a cationic membrane and stained with Coomassie Blue. Following staining, the membranes are washed and sections thought to contain the unknown polypeptides are cut out and dissected into small pieces. The membranes are placed in 500 µl microcentrifuge tubes and immersed in 10 to 20 µl of proteolytic digestion buffer (100 mM Tris-HCl, pH 8.2, containing 0.1 M NaCl, 10% acetonitrile,
15 2 mM CaCl₂ and 5 µg/ml trypsin) (Sigma, St. Louis, MO). After 15 hr at 37°C, 3 µl of saturated urea and 1 µl of 100 µg/ml trypsin are added and incubated for an additional 5 hr at 37°C. The digestion mixture is acidified with 3 µl of 10% trifluoroacetic acid and centrifuged to separate supernatant from membrane. The supernatant is injected directly onto a microbore, reverse phase HPLC column and eluted with a linear gradient of
20 acetonitrile in 0.05% trifluoroacetic acid. The eluate is fed directly into an electrospray mass spectrometer, after passing through a stream splitter if necessary to adjust the volume of material. The data is analyzed following the procedures set forth in Example 12, Section A.

25 Example 13: Gene Immunization Protocol

A. In Vivo Antigen Expression. Gene immunization circumvents protein purification steps by directly expressing an antigen in vivo after inoculation of the appropriate expression vector. Also, production of antigen by this method may allow correct protein folding and glycosylation since the protein is produced in mammalian
30 tissue. The method utilizes insertion of the gene sequence into a plasmid which contains a CMV promoter, expansion and purification of the plasmid and injection of the plasmid DNA into the muscle tissue of an animal. Preferred animals include mice and rabbits. See, for example, H. Davis et al., Human Molecular Genetics 2:1847-1851 (1993). After one or two booster immunizations, the animal can then be bled,
35 ascites fluid collected, or the animal's spleen can be harvested for production of hybridomas.

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B. Plasmid Preparation and Purification. The CS194 cDNA sequences are generated from the CS194 cDNA-containing vector using appropriate PCR primers containing suitable 5' restriction sites following the procedures described in Example 11. The PCR product is cut with appropriate restriction enzymes and inserted into a vector which contains the CMV promoter (for example, pRc/CMV or pcDNA3 vectors from Invitrogen, San Diego, CA). This plasmid then is expanded in the appropriate bacterial strain and purified from the cell lysate using a CsCl gradient or a Qiagen plasmid DNA purification column. All these techniques are familiar to one of ordinary skill in the art of molecular biology.

C. Immunization Protocol. Anesthetized animals are immunized intramuscularly with 0.1-100 µg of the purified plasmid diluted in PBS or other DNA uptake enhancers (Cardiotoxin, 25% sucrose). See, for example, H. Davis et al, Human Gene Therapy 4:733-740 (1993); and P. W. Wolff et al, Biotechniques 11:474-485 (1991). One to two booster injections are given at monthly intervals.

D. Testing and Use of Antiserum. Animals are bled and the resultant sera tested for antibody using peptides synthesized from the known gene sequence (see Example 16) using techniques known in the art, such as Western blotting or EIA techniques. Antisera produced by this method can then be used to detect the presence of the antigen in a patient's tissue or cell extract, or in a patient's serum, by ELISA or Western blotting techniques, such as those described in Examples 15 through 18.

Example 14: Production of Antibodies Against CS194

A. Production of Polyclonal Antisera. Antiserum against CS194 was prepared by injecting rabbits with peptides whose sequences were derived from that of the predicted amino acid sequence of the CS194 consensus sequence (SEQUENCE ID NO 20). The synthesis of these CS194 peptides is described in Example 10. Peptides used as immunogens were not conjugated to a carrier such as keyhole limpet hemocyanine, KLH, (i.e., they were unconjugated.).

Animal Immunization. Female white New Zealand rabbits weighing 2 kg or more were used for raising polyclonal antiserum. One animal was immunized per unconjugated peptide (SEQUENCE ID NOS 42-49). One week prior to the first immunization, blood samples (5 to 10 ml) were obtained from the animals to serve as non-immune prebleed samples.

Unconjugated peptides, SEQUENCE ID NOS 42-49, were used to prepare the primary immunogen by emulsifying 0.5 ml of the peptide at a concentration of 2 mg/ml

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in PBS (pH 7.2) which contained 0.5 ml of complete Freund's adjuvant (CFA) (Difco, Detroit, MI). The immunogen was injected into several sites of the animal via subcutaneous, intraperitoneal, and intramuscular routes of administration. Four weeks following the primary immunization, a booster immunization was administered. The immunogen used for the booster immunization dose was prepared by emulsifying 0.5 ml of the same unconjugated peptide used for the primary immunogen, except that the peptide now was diluted to 1 mg/ml with 0.5 ml of incomplete Freund's adjuvant (IFA) (Difco, Detroit, MI). Again, the booster dose was administered into several sites via subcutaneous, intraperitoneal and intramuscular types of injections. The animals were bled (5 ml) two weeks after the booster immunizations and each serum was tested for immunoreactivity to the peptide as described below. The booster and bleed schedule were repeated at 4 week intervals until an adequate titer was obtained. The titer or concentration of antiserum was determined using unconjugated peptides in a microtiter EIA as described in Example 17, below. An antibody titer of 1:500 or greater was considered an adequate titer for further use and study.

Table 1. Titer of rabbit anti-CS194 peptide antisera (13 week bleed)

Peptide Immunogen		Titer
CS194.1	SEQUENCE ID NO 45	37,000
CS194.2	SEQUENCE ID NO 46	7,300
CS194.3	SEQUENCE ID NO 47	<100
CS194.4	SEQUENCE ID NO 48	25,000

B. Production of Monoclonal Antibody.

1. Immunization Protocol. Mice are immunized using peptides which can either be conjugated to a carrier such as KLH [prepared as described hereinbelow, or unconjugated (i.e., not conjugated to a carrier such as KLH)] except that the amount of the unconjugated or conjugated peptide for monoclonal antibody production in mice is one-tenth the amount used to produce polyclonal antisera in rabbits. Thus, the primary immunogen consists of 100 µg of unconjugated or conjugated peptide in 0.1 ml of CFA emulsion; while the immunogen used for booster immunizations consists of 50 µg of unconjugated or conjugated peptide in 0.1 ml of IFA. Hybridomas for the generation of monoclonal antibodies are prepared and screened using standard techniques. The methods used for monoclonal antibody development follow procedures known in the art such as those detailed in Kohler and Milstein, Nature

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256:494 (1975) and reviewed in J.G.R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL (1982). Another method of monoclonal antibody development which is based on the Kohler and Milstein method is that of L.T. Mimms et al., Virology 176:604-619 (1990), which is incorporated herein by reference.

The immunization regimen (per mouse) consists of a primary immunization with additional booster immunizations. The primary immunogen used for the primary immunization consists of 100 µg of unconjugated or conjugated peptide in 50 µl of PBS (pH 7.2) previously emulsified in 50 µl of CFA. Booster immunizations performed at approximately two weeks and four weeks post primary immunization consist of 50 µg of unconjugated or conjugated peptide in 50 µl of PBS (pH 7.2) emulsified with 50 µl IFA. A total of 100 µl of this immunogen is inoculated intraperitoneally and subcutaneously into each mouse. Individual mice are screened for immune response by microtiter plate enzyme immunoassay (EIA) (as described in Example 17) approximately four weeks after the third immunization. Mice are inoculated either intravenously, intrasplenically or intraperitoneally with 50 µg of unconjugated or conjugated peptide in PBS (pH 7.2) approximately fifteen weeks after the third immunization..

Three days after this intravenous boost, splenocytes are fused with, for example, Sp2/0-Ag14 myeloma cells (Milstein Laboratories, England) using the polyethylene glycol (PEG) method. The fusions are cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% fetal calf serum (FCS), plus 1% hypoxanthine, aminopterin and thymidine (HAT). Bulk cultures are screened by microtiter plate EIA following the protocol in Example 17. Clones reactive with the peptide used an immunogen and non-reactive with other peptides (i.e., peptides of CS194 not used as the immunogen) are selected for final expansion. Clones thus selected are expanded, aliquoted and frozen in IMDM containing 10% FCS and 10% dimethyl-sulfoxide, (DMSO).

2. Peptide Conjugation. Peptide is conjugated to maleimide activated KLH (commercially available as Imject®, available from Pierce Chemical Company, Rockford, IL). Imject® contains about 250 moles of reactive maleimide groups per mole of hemocyanine. The activated KLH is dissolved in phosphate buffered saline (PBS, pH 8.4) at a concentration of about 7.7 mg/ml. The peptide is conjugated through cysteines occurring in the peptide sequence, or to a cysteine previously added to the synthesized peptide in order to provide a point of attachment. The peptide is dissolved in DMSO (Sigma Chemical Company, St. Louis, MO) and reacted with the

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activated KLH at a mole ratio of about 1.5 moles of peptide per mole of reactive maleimide attached to the KLH. A procedure for the conjugation of peptide is provided hereinbelow. It is known to the ordinary artisan that the amounts, times and conditions of such a procedure can be varied to optimize peptide conjugation.

5 The conjugation reaction described hereinbelow is based on obtaining 3 mg of KLH peptide conjugate ("conjugated peptide"), which contains about 0.77 μ moles of reactive maleimide groups. This quantity of peptide conjugate usually is adequate for one primary injection and four booster injections for production of polyclonal antisera in a rabbit. Briefly, peptide is dissolved in DMSO at a concentration of 1.16 μ moles/100 μ l of DMSO. One hundred microliters (100 μ l) of the DMSO solution are added to 380 μ l of the activated KLH solution prepared as described hereinabove, and 20 μ l of PBS (pH 8.4) are added to bring the volume to 500 μ l. The reaction is incubated overnight at room temperature with stirring. The extent of reaction is determined by measuring the amount of unreacted thiol in the reaction mixture. The difference between the starting concentration of thiol and the final concentration is assumed to be the concentration of peptide which has coupled to the activated KLH. The amount of remaining thiol is measured using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), Pierce Chemical Company, Rockford, IL). Cysteine standards are made at a concentration of 0, 0.1, 0.5, 2, 5 and 20 mM by dissolving 35 mg of cysteine HCl (Pierce Chemical Company, Rockford, IL) in 10 ml of PBS (pH 7.2) and diluting the stock solution to the desired concentration(s). The photometric determination of the concentration of thiol is accomplished by placing 200 μ l of PBS (pH 8.4) in each well of an Immulon 2[®] microwell plate (Dynex Technologies, Chantilly, VA). Next, 10 μ l of standard or reaction mixture are added to each well. Finally, 20 μ l of Ellman's reagent at a concentration of 1 mg/ml in PBS (pH 8.4) are added to each well. The wells are incubated for 10 minutes at room temperature, and the absorbance of all wells is read at 415 nm with a microplate reader (such as the BioRad Model 3550, BioRad, Richmond, CA). The absorbance of the standards is used to construct a standard curve and the thiol concentration of the reaction mixture is determined from the standard curve. A decrease in the concentration of free thiol is indicative of a successful conjugation reaction. Unreacted peptide is removed by dialysis against PBS (pH 7.2) at room temperature for 6 hours. The conjugate is stored at 2-8°C if it is to be used immediately; otherwise, it is stored at -20°C or colder.

3. Production of Ascites Fluid Containing Monoclonal Antibodies.

35 Frozen hybridoma cells prepared as described hereinabove are thawed and placed into expansion culture. Viable hybridoma cells are inoculated intraperitoneally into Pristane

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treated mice. Ascitic fluid is removed from the mice, pooled, filtered through a 0.2 μ filter and subjected to an immunoglobulin class G (IgG) analysis to determine the volume of the Protein A column required for the purification.

4. Purification of Monoclonal Antibodies From Ascites Fluid. Briefly,
- 5 filtered and thawed ascites fluid is mixed with an equal volume of Protein A sepharose binding buffer (1.5 M glycine, 3.0 M NaCl, pH 8.9) and refiltered through a 0.2 μ filter. The volume of the Protein A column is determined by the quantity of IgG present in the ascites fluid. The eluate then is dialyzed against PBS (pH 7.2) overnight at 2-8°C. The dialyzed monoclonal antibody is sterile filtered and dispensed in aliquots.
- 10 The immunoreactivity of the purified monoclonal antibody is confirmed by determining its ability to specifically bind to the peptide used as the immunogen by use of the EIA microtiter plate assay procedure of Example 17. The specificity of the purified monoclonal antibody is confirmed by determining its lack of binding to irrelevant peptides such as peptides of CS194 not used as the immunogen. The purified anti-
- 15 CS194 monoclonal thus prepared and characterized is placed at either 2-8°C for short term storage or at -80°C for long term storage.

5. Further Characterization of Monoclonal Antibody. The isotype and subtype of the monoclonal antibody produced as described hereinabove can be determined using commercially available kits (available from Amersham. Inc.,
- 20 Arlington Heights, IL). Stability testing also can be performed on the monoclonal antibody by placing an aliquot of the monoclonal antibody in continuous storage at 2-8°C and assaying optical density (OD) readings throughout the course of a given period of time.

- C. Use of Recombinant Proteins as Immunogens. It is within the scope of the
- 25 present invention that recombinant proteins made as described herein can be utilized as immunogens in the production of polyclonal and monoclonal antibodies, with corresponding changes in reagents and techniques known to those skilled in the art.

30 Example 15: Purification of Serum Antibodies Which Specifically
Bind to CS194 Peptides

- Immune sera, obtained as described hereinabove in Examples 13 and/or 14, is affinity purified using immobilized synthetic peptides prepared as described in Example 10, or recombinant proteins prepared as described in Example 11. An IgG fraction of the antiserum is obtained by passing the diluted, crude antiserum over a Protein A
- 35 column (Affi-Gel protein A, Bio-Rad, Hercules, CA). Elution with a buffer (Binding Buffer, supplied by the manufacturer) removes substantially all proteins that are not

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immunoglobulins. Elution with 0.1M buffered glycine (pH 3) gives an immunoglobulin preparation that is substantially free of albumin and other serum proteins.

Immunoaffinity chromatography is performed to obtain a preparation with a higher fraction of specific antigen-binding antibody. The peptide used to raise the antiserum is immobilized on a chromatography resin, and the specific antibodies directed against its epitopes are adsorbed to the resin. After washing away non-binding components, the specific antibodies are eluted with 0.1 M glycine buffer, pH 2.3. Antibody fractions are immediately neutralized with 1.0 M Tris buffer (pH 8.0) to preserve immunoreactivity. The chromatography resin chosen depends on the reactive groups present in the peptide. If the peptide has an amino group, a resin such as Affi-Gel 10 or Affi-Gel 15 is used (Bio-Rad, Hercules, CA). If coupling through a carboxy group on the peptide is desired, Affi-Gel 102 can be used (Bio-Rad, Hercules, CA). If the peptide has a free sulfhydryl group, an organomercurial resin such as Affi-Gel 501 can be used (Bio-Rad, Hercules, CA).

Alternatively, spleens can be harvested and used in the production of hybridomas to produce monoclonal antibodies following routine methods known in the art as described hereinabove.

Example 16: Western Blotting of Tissue Samples

Protein extracts were prepared by homogenizing tissue samples in 0.1M Tris-HCl (pH 7.5), 15% (w/v) glycerol, 0.2 mM EDTA, 1.0 mM 1,4-dithiothreitol, 10 µg/ml leupeptin and 1.0 mM phenylmethylsulfonylfluoride (S. R. Kain et al., Biotechniques 17:982 (1994)). Following homogenization, the homogenates were centrifuged at 4°C for 5 minutes to separate supernatant from debris. For protein quantitation, 3-10 µl of supernatant were added to 1.5 ml of bicinchoninic acid reagent (Sigma, St. Louis, MO), and the resulting absorbance at 562 nm were measured.

For SDS-PAGE, samples were adjusted to desired protein concentration with Tricine Buffer (Novex, San Diego, CA), mixed with an equal volume of 2X Tricine sample buffer (Novex, San Diego, CA), and heated for 5 minutes at 100°C in a thermal cycler. Samples were then applied to a Novex 10-20% Precast Tricine Gel for electrophoresis. Following electrophoresis samples were transferred from the gels to nitrocellulose membranes in Novex Tris-Glycine Transfer buffer. Membranes were then probed with specific anti-peptide antibodies using the reagents and procedures provided in the Western Lights Plus or Western Lights (Tropix, Bedford, MA)

chemiluminescence detection kits. Chemiluminescent bands were visualized by exposing the developed membranes to Hyperfilm ECL (Amersham, Arlington Heights, IL).

Figure 3 shows the results of the Western blot performed on a panel of tissue extracts using antiserum against the CS194 synthetic peptide of SEQUENCE ID NO 48 (see Example 14). Each lane of Figure 3 represents a different tissue protein extract : (1) prostate; (2) breast; (3) bladder; (4-5) lung; (6-7) colon cancer; (7-9) colon; (10) blank; and (12) markers). A broad band between 60 kD and 148 kD (see arrows) was observed in all three normal colon tissue extracts (lanes 7-9), while a slightly lower molecular weight broad band (see arrows) was observed in one of two colon cancer tissues (lane 5). Since CS194 has eight potential N-glycosylation sites, the mobility difference between the two bands was most likely due to glycosylation. The broadness of the bands was consistent with the presence of glycosylation.

Competition experiments were carried out in an analogous manner as above, with the following exception: the primary antibodies (anti-peptide polyclonal antisera) were pre-incubated for 30 minutes at room temperature with varying concentrations of peptide immunogen prior to exposure to the nitrocellulose filter. Development of the Western blot was continued as above. Antibody binding to the band between 60 and 148 kD was inhibited at a concentration of 2.4 μ M of CS194 synthetic peptide (SEQUENCE ID NO 48).

After visualization of the bands on film, the bands were also visualized directly on the membranes by the addition and development of chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP). This chromogenic solution contains 0.016% BCIP in a solution containing 100 mM NaCl, 5 mM $MgCl_2$ and 100 mM Tris-HCl, pH 9.5. The filter was incubated in the solution at room temperature until the bands developed to the desired intensity. Molecular mass determination was made based upon the mobility of pre-stained molecular weight standards (Novex, San Diego, CA) and biotinylated molecular weight standards (Tropix, Bedford, MA).

Example 17: EIA Microtiter Plate Assay

The immunoreactivity of antiserum obtained from rabbits as described in Example 14 was determined by means of a microtiter plate EIA, as follows. Briefly, synthetic peptides, SEQUENCE ID NOS 42-49, prepared as described in Example 10, were dissolved in carbonate buffer (50 mM, pH 9.6) to a final concentration of 2 μ g/ml. Next, 100 μ l of the peptide or protein solution were placed in each well of an Immulon 2[®] microtiter plate (Dynex Technologies, Chantilly, VA). The plate was incubated overnight at room temperature and then washed four times with deionized

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water. The wells were blocked by adding 125 µl of a suitable protein blocking agent, such as Superblock® (Pierce Chemical Company, Rockford, IL), to each well and then immediately discarding the solution. This blocking procedure was performed three times. Antiserum obtained from immunized rabbits or mice, prepared as previously described, was diluted in a protein blocking agent (e.g., a 3% Superblock® solution) in PBS containing 0.05% Tween-20® (monolaurate polyoxyethylene ether) (Sigma Chemical Company, St. Louis, MO) and 0.05% sodium azide at dilutions of 1:100, 1:500, 1:2500, 1:12,500, and 1:62,500 and placed in each well of the coated microtiter plate. The wells then were incubated for three hours at room temperature. Each well was washed four times with deionized water. One hundred microliters of alkaline phosphatase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG antiserum (Southern Biotech, Birmingham, AB) diluted 1:2000 in 3% Superblock® solution in phosphate buffered saline containing 0.05% Tween 20® and 0.05% sodium azide, were added to each well. The wells were incubated for two hours at room temperature. Next, each well was washed four times with deionized water. One hundred microliters of paranitrophenyl phosphate substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) then were added to each well. The wells were incubated for thirty minutes at room temperature. The absorbance at 405 nm was read in each well. Positive reactions were identified by an increase in absorbance at 405 nm in the test well above that absorbance given by a non-immune serum (negative control). A positive reaction was indicative of the presence of detectable anti-CS194 antibodies. Titers of the anti-peptide antisera were calculated from the previously described dilutions of antisera and defined as the calculated dilution, where $A_{405nm}=0.5$ OD.

Example 18: Coating of Solid Phase Particles

A. Coating of Microparticles with Antibodies Which Specifically Bind to CS194 Antigen. Affinity purified antibodies which specifically bind to CS194 protein (see Example 15) are coated onto microparticles of polystyrene, carboxylated polystyrene, polymethylacrylate or similar particles having a radius in the range of about 0.1 to 20 µm. Microparticles may be either passively or actively coated. One coating method comprises coating EDAC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Aldrich Chemical Co., Milwaukee, WI) activated carboxylated latex microparticles with antibodies which specifically bind to CS194 protein, as follows. Briefly, a final 0.375% solid suspension of resin washed carboxylated latex microparticles (available from Bangs Laboratories, Carmel, IN or Serodyn, Indianapolis, IN) are mixed in a solution containing 50 mM MES buffer, pH

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4.0 and 150 mg/l of affinity purified anti-CS194 antibody (see Example 14) for 15 min in an appropriate container. EDAC coupling agent is added to a final concentration of 5.5 µg/ml to the mixture and mixed for 2.5 hr at room temperature.

5 The microparticles then are washed with 8 volumes of a Tween 20®/sodium phosphate wash buffer (pH 7.2) by tangential flow filtration using a 0.2 µm Microgon Filtration module. Washed microparticles are stored in an appropriate buffer which usually contains a dilute surfactant and irrelevant protein as a blocking agent, until needed.

10 B. Coating of 1/4 Inch Beads. Antibodies which specifically bind to CS194- antigen also may be coated on the surface of 1/4 inch polystyrene beads by routine methods known in the art (Snitman et al, US Patent 5,273,882, incorporated herein by reference) and used in competitive binding or EIA sandwich assays.

15 Polystyrene beads first are cleaned by ultrasonicing them for about 15 seconds in 10 mM NaHCO₃ buffer at pH 8.0. The beads then are washed in deionized water until all fines are removed. Beads then are immersed in an antibody solution in 10 mM carbonate buffer, pH 8 to 9.5. The antibody solution can be as dilute as 1 µg/ml in the case of high affinity monoclonal antibodies or as concentrated as about 500 µg/ml for polyclonal antibodies which have not been affinity purified. Beads are coated for at least 12 hours at room temperature, and then they are washed with deionized water.
20 Beads may be air dried or stored wet (in PBS, pH 7.4). They also may be overcoated with protein stabilizers (such as sucrose) or protein blocking agents used as non-specific binding blockers (such as irrelevant proteins, Carnation skim milk, Superblock®, or the like).

25 Example 19: Microparticle Enzyme Immunoassay (MEIA)

CS194 antigens are detected in patient test samples by performing a standard antigen competition EIA or antibody sandwich EIA and utilizing a solid phase such as microparticles (MEIA). The assay can be performed on an automated analyzer such as the IMx® Analyzer (Abbott Laboratories, Abbott Park, IL).

30 A. Antibody Sandwich EIA. Briefly, samples suspected of containing CS194 antigen are incubated in the presence of anti-CS194 antibody-coated microparticles (prepared as described in Example 17) in order to form antigen/antibody complexes. The microparticles then are washed and an indicator reagent comprising an antibody conjugated to a signal generating compound (i.e., enzymes such as alkaline
35 phosphatase or horseradish peroxidase) is added to the antigen/antibody complexes or the microparticles and incubated. The microparticles are washed and the bound

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antibody/antigen/antibody complexes are detected by adding a substrate (e.g., 4-methyl umbelliferyl phosphate (MUP), or OPD/peroxide, respectively), that reacts with the signal generating compound to generate a measurable signal. An elevated signal in the test sample, compared to the signal generated by a negative control, detects the presence of CS194 antigen. The presence of CS194 antigen in the test sample is indicative of a diagnosis of a GI tract disease or condition, such as GI tract cancer.

B. Competitive Binding Assay. The competitive binding assay uses a peptide or protein that generates a measurable signal when the labeled peptide is contacted with an anti-peptide antibody coated microparticle. This assay can be performed on the IMx[®] Analyzer (available from Abbott Laboratories, Abbott Park, IL). The labeled peptide is added to the CS194 antibody-coated microparticles (prepared as described in Example 17) in the presence of a test sample suspected of containing CS194 antigen, and incubated for a time and under conditions sufficient to form labeled CS194 peptide (or labeled protein) / bound antibody complexes and/or patient CS194 antigen / bound antibody complexes. The CS194 antigen in the test sample competes with the labeled CS194 peptide (or CS194 protein) for binding sites on the microparticle. CS194 antigen in the test sample results in a lowered binding of labeled peptide and antibody coated microparticles in the assay since antigen in the test sample and the CS194 peptide or CS194 protein compete for antibody binding sites. A lowered signal (compared to a control) indicates the presence of CS194 antigen in the test sample. The presence of CS194 antigen suggests the diagnosis of a GI tract disease or condition, such as GI tract cancer.

The CS194 polynucleotides and the proteins encoded thereby which are provided and discussed hereinabove are useful as markers of GI tract tissue disease, especially GI tract cancer. Tests based upon the appearance of this marker in a test sample such as blood, plasma or serum can provide low cost, non-invasive, diagnostic information to aid the physician to make a diagnosis of cancer, to help select a therapy protocol, or to monitor the success of a chosen therapy. This marker may appear in readily accessible body fluids such as blood, urine or stool as antigens derived from the diseased tissue which are detectable by immunological methods. This marker may be elevated in a disease state, altered in a disease state, or be a normal protein of the GI tract which appears in an inappropriate body compartment.

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SEQUENCE LISTING

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(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/829,754
(B) FILING DATE: 31-MAR-1997
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 223 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAAATCACAG	GGAGATGTAC	AGCAATGGGG	CCATTTAAGA	GTTCTGTGTT	CATCTTGATT	60
CTTCACCTTC	TAGAAGGGGC	CCTGAGTAAT	TCACTCATTTC	AGCTGAACAA	CAATGGCTAT	120
GAAGGCATTG	TCGTTGCAAT	CGACCCCAAT	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	180
ATAAAGGACA	TGGTGACCCA	GGCATCTCTG	TATCTGTTTG	AAG		223

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 273 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: base_polymorphism
- (B) LOCATION: 158
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:

- (A) NAME/KEY: base_polymorphism
- (B) LOCATION: 221
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:

- (A) NAME/KEY: base_polymorphism
- (B) LOCATION: 231
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:

- (A) NAME/KEY: base_polymorphism
- (B) LOCATION: 251
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTGCAATCG	ACCCCAATGT	GCCAGAAGAT	GAAACACTCA	TTCAACAAAT	AAAGGACATG	60
GTGACCCAGG	CATCTCTGTA	TCTGTTTGAA	GCTACAGGAA	AGCGATTTTA	TTTCAAAAAT	120
GTTGCCATTT	TGATTCCCTGA	AACATGGAAG	ACAAAGGNTG	ACTATGTGAG	ACCAAAACTT	180
GAGACCTACA	AAAATGCTGA	TGTTCTGGTT	GCTGAGTCTA	NTCCTCCAGG	NAATGATGAA	240
CCCTACACTG	NGCAGATGGG	CAACTGTGGC	GAG			273

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 240 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

09049696.032798

AAAATGCTGA	TGTTCTGGTT	GCTGAGTCTA	CTCCTCCAGG	TAATGATGAA	CCCTACACTG	60
AGCAGATGGG	CAACTGTGGA	GAGAAGGGTG	AAAGGATCCA	CCTCACTCCT	GATTTCATTG	120
CAGGAAAAAA	GTTAGCTGAA	TATGGACCAC	AAGGTAGGGC	ATTTGTCCAT	GAGTGGGCTC	180
ATCTACGATG	GGGAGTATTT	GACGAGTACA	ATAATGATGA	GAAATTCTAC	TTATCCAATG	240

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 181 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAAAAGATGC	ACATTCAATA	AAGTAACAGG	ACTCTATGAA	AAAGGATGTG	AGTTTGTCT	60
CCAATCCCGC	CAGACGGAGA	AGGCTTCTAT	AATGTTTGCA	CAACATGTTG	ATTCTATAGT	120
TGAATTCTGT	ACAGAACAAA	ACCACAACAA	AGAAGCTCCA	AACAAGCAAA	ATCAAAAATG	180
C						181

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTATAGTTGA	ATTCTGTACA	GAACAAAACC	ACAACAAAGA	AGCTCCAAAC	AAGCAAAATC	60
AAAAATGCAA	TCTCCGAAGC	ACATGGGAAG	TGATCCGTGA	TTCTGAGGAC	T'TAAGAAAA	120
CCACTCCTAT	GACAACACAG	CCACCAAATC	CCACCTTCTC	ATTGCTGCAG	ATTGGACAAA	180
GAATTGTGTG	TTTAGTCCTT	GACAAATCTG	GAAGCATGGC			220

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 252 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAAGAATTG	TGTGTTTAGT	CCTTGACAAA	TCTGGAAGCA	TGGCGACTGG	TAACCGCCTC	60
AATCGACTGA	ATCAAGCAGG	CCAGCTTTTC	CTGCTGCAGA	CAGTTGAGCT	GGGGTCCTGG	120
GTTGGGATGG	TGACATTTGA	CAGTGCTGCC	CATGTACAAA	GTGAATCAT	ACAGATAAAC	180
AGTGGCAGTG	ACAGGGACAC	ACTCGCCAAA	AGATTACCTG	CAGCAGCTTC	AGGAGGGACG	240
TCCATCTGCA	GC					252

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 289 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: base_polymorphism
- (B) LOCATION: 92

05049696.032798

(D) OTHER INFORMATION: /note= ``N' represents an A or G or T or C polymorphism at this position"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAAATATCCA	ACTGATGGAT	CTGAAATTGT	GCTGCTGACG	GATGGGGAAG	ACAACACTAT	60
AAGTGGGTGC	TTTAACGAGG	TCAAACAAAG	TNGTGCCATC	ATCCACACAG	TCGCTTTGGG	120
GCCCTCTGCA	GCTCAAGAAC	TAGAGGAGCT	GTCCAAAATG	ACAGGAGGTT	TACAGACATA	180
TGCTTCAGAT	CAAGTTCAGA	ACAATGGCCT	CATTGATGCT	TTTGGGGCCC	TTTCATCAGG	240
AAATGGAGCT	GTCTCTCAGC	GCTCCATCCA	GCTTGAGAGT	AAGGGATTA		289

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 253 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT	CAAGAACTAG	60
AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC	TTCAGATCAA	GTTTCAGAACA	120
ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	180
CCATCCAGCT	TGAGAGTAAG	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	240
TGATCGTGA	CAG					253

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 218 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAACAATGGC	CTCATTGATG	CTTTTGGGGC	CCTTTCATCA	GGAAATGGAG	CTGTCTCTCA	60
GCGCTCCATC	CAGCTTGAGA	GTAAGGGATT	AACCCTCCAG	AACAGCCAGT	GGATGAATGG	120
CACAGTGATC	GTGGACAGCA	CCGTGGGAAA	GGACACTTTG	TTTCTTATCA	CCTGGACAAC	180
GCAGCCTCCC	CAAATCCTTC	TCTGGGATCC	CAGTGGAC			218

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 229 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCACAGTGA	TCGTGGACAG	CACCGTGGGA	AAGGACACTT	TGTTTCTTAT	CACCTGGACA	60
ACGCAGCCTC	CCCAAATCCT	TCTCTGGGAT	CCCAGTGGAC	AGAAGCAAGG	TGGCTTTGTA	120
GTGGACAAAA	ACACCAAAAT	GGCCTACCTC	CAAATCCCAG	GCATTGCTAA	GGTTGGCACT	180
TGGAAATACA	GTCTGCAAGC	AAGCTCACAA	ACCTTGACCC	TGACTGTCA		229

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGCTTTGTAG TGGACAAAAA CACCAAAATG GCCTACCTCC AAATCCCAGG CATTGCTAAG	60
GTTGGCACTT GGAAATACAG TCTGCAAGCA AGCTCACAAA CCTTGACCCT GACTGTCACG	120
TCCCGTGCCT CCAATGCTAC CTGCCTCCA ATTACAGTGA CTTCCAAAAC GAACAAGGAC	180
ACCAGCAAAT T	191

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 235 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACACCAGCA AATTCCCCAG CCCTCTGGTA GTTTATGCAA ATATTCGCCA AGGAGCCTCC	60
CCAATTCTCA GGGCCAGTGT CACAGCCCTG ATTGAATCAG TGAATGGAAA AACAGTTACC	120
TTGGAACTAC TGGATAATGG AGCAGGTGCT GATGCTACTA AGGATGACGG TGTCTACTCA	180
AGGTATTTCA CAACTTATGA CACGAATGGT AGATACAGTG TAAAAGTGCG GGCTC	235

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 227 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTGCGGGCTC TGGGAGGAGT TAACGCAGCC AGACGGAGAG TGATACCCCA GCAGAGTGGA	60
GCACTGTACA TACCTGGCTG GATTGAGAAT GATGAAATAC AATGGAATCC ACCAAGACCT	120
GAAATTAATA AGGATGATGT TCAACACAAG CAAGTGTGTT TCAGCAGAAC ATCCTCGGGA	180
GGCTCATTTG TGGCTTCTGA TGTCCCAAAT GCTCCCATAC CTGATCT	227

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 248 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACCTGAAGGC GGAAATTCAC GGGGGCAGTC TCATTAATCT GACTTGGACA GCTCCTGGGG	60
ATGATTATGA CCATGGAACA GCTCACAAGT ATATCATTCG AATAAGTACA AGTATTCTTG	120
ATCTCAGAGA CAAGTTCAAT GAATCTCTTC AAGTGAATAC TACTGCTCTC ATCCCAAAGG	180
AAGCCAACTC TGAGGAAGTC TTTTGTGTTA AACCAGAAAA CATTACTTTT GAAAATGGCA	240
CAGATCTT	248

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

00049660 "96964060

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ACAGATCTTT TCATTGCTAT TCAGGCTGTT GATAAGGTCG ATCTGAAATC AGAAATATCC	60
AACATTGCAC GAGTATCTTT GTTTATTCTT CCACAGACTC CGCCAGAGAC ACCTAGTCCT	120
GATGAAACGT CTGCTCCTTG TCCTAATATT CATA	154

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 242 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCTATTCTT CCACAGACTC CGCCAGAGAC ACCTAGTCCT GATGAAACGT CTGCTCCTTG	60
TCCTAATATT CATATCAACA GCACCATTC TGGCATTAC ATTTTAAAAA TTATGTGGAA	120
GTGGATAGGA GAACTGCAGC TGTCATAGC CTAGGGCTGA ATTTTGTCA GATAAATAAA	180
ATAAATCATT CATCTTTTTT TTTGATTATA AAATTTTCTA AAATGTATTT TAGACTTCCT	240
GT	242

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGCATTACACA TTTTAAAAAT TATGTGGAAG TGGATAGGAG AACTGCAGCT GTCAATAGCC	60
TAGGGCTGAA TTTTGTGTCAG ATAAATAAAA TAAATCATTC ATCCTT	106

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2813 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAAATCACAG GGAGATGTAC AGCAATGGGG CCATTTAAGA GTTCTGTGTT CATCTTGATT	60
CTTCACCTTC TAGAAGGGGC CCTGAGTAAT TCACTCATTC AGCTGAACAA CAATGGCTAT	120
GAAGGCATTG TCGTTGCAAT CGACCCCAAT GTGCCAGAAG ATGAAACACT CATTCACAA	180
ATAAAGGACA TGGTGACCCA GGCATCTCTG TATCTGTTTG AAGCTACAGG AAAGCGATTT	240
TATTTCAAAA ATGTTGCCAT TTTGATTCTT GAAACATGGA AGACAAAGGC TGACTATGTG	300
AGACCAAAAC TTGAGACCTA CAAAAATGCT GATGTTCTGG TTGCTGAGTC TACTCCTCCA	360
GGTAATGATG AACCCTACAC TGAGCAGATG GGCAACTGTG GAGAGAAGGG TGAAAGGATC	420
CACCTCACTC CTGATTTTCAT TGCAGGAAAA AAGTTAGCTG AATATGGACC ACAAGGTAGG	480
GCATTTGTCC ATGAGTGGGC TCATCTACGA TGGGGAGTAT TTGACGAGTA CAATAATGAT	540
GAGAAATTC TACTTATCCA TGGAAGAATA CAAGCAGTAA GATGTTTCAGC AGGTATTACT	600
GGTACAAATG TAGTAAAGAA GTGTGAGGGA GGCAGCTGTT ACACCAAAAG ATGCACATTC	660
AATAAGTAA CAGGACTCTA TGAAAAAGGA TGTGAGTTTG TTCTCCAATC CCGCCAGACG	720
GAGAAGGCTT CTATAATGTT TGCACAACAT GTTGATTCTA TAGTTGAATT CTGTACAGAA	780
CAAAACCACA ACAAAGAAGC TCCAAACAAG CAAAATCAAA AATGCAATCT CCGAAGCACA	840
TGGGAAGTGA TCCGTGATTC TGAGGACTTT AAGAAAACCA CTCCTATGAC AACACAGCCA	900
CCAAATCCCA CCTTCTCATT GCTGCAGATT GGACAAAGAA TTGTGTGTTT AGTCCTTGAC	960

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AAATCTGGAA	GCATGGCGAC	TGGTAACCGC	CTCAATCGAC	TGAATCAAGC	AGGCCAGCTT	1020
TTCCTGCTGC	AGACAGTTGA	GCTGGGGTCC	TGGGTTGGGA	TGGTGACATT	TGACAGTGCT	1080
GCCCATGTAC	AAAGTGAAC	CATACAGATA	AACAGTGGCA	GTGACAGGGA	CACACTCGCC	1140
AAAAGATTAC	CTGCAGCAGC	TTCAGGAGGG	ACGTCCATCT	GCAGCGGGCT	TCCGATCGGCA	1200
TTTACTGTGA	TTAGGAAGAA	ATATCCAAC	GATGGATCTG	AAATTGTGCT	GCTGACGGAT	1260
GGGGAAGACA	ACACTATAAG	TGGGTGCTTT	AACGAGGTCA	AACAAAGTGG	TGCCATCATC	1320
CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	1380
GGAGGTTTAC	AGACATATGC	TTCAGATCAA	GTTTCAGAAC	ATGGCCTCAT	TGATGCTTTT	1440
GGGGCCCTTT	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG	1500
GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA	CAGCACCGTG	1560
GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC	CTCCCCAAAT	CCTTCTCTGG	1620
GATCCCAGTG	GACAGAAGCA	AGGTGGCTTT	GTAAGTGGCA	AAAACACCAA	AATGGCCTAC	1680
CTCCAAATCC	CAGGCATTGC	TAAGGTTGGC	ACTTGGAAAT	ACAGTCTGCA	AGCAAGCTCA	1740
CAAACCTTGA	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA	1800
GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	GCCCTCTGGT	AGTTTATGCA	1860
AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG	TCACAGCCCT	GATTGAATCA	1920
GTGAATGGAA	AAACAGTTAC	CTTGGAACTA	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	1980
AAGGATGACG	GTGTCTACTC	AAGGTATTTT	ACAACCTTAT	ACACGAATGG	TAGATACAGT	2040
GTAAAAGTGC	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCCGAGCAG	2100
AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG	GAATCCACCA	2160
AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG	TGTGTTTCAG	CAGAACATCC	2220
TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC	CCAAATGCTC	CCATACCTGA	TCTCTTCCCA	2280
CCTGGCCAAA	TCACCGACCT	GAAGGCGGAA	ATTACCGGGG	GCAGTCTCAT	TAATCTGACT	2340
TGGACAGCTC	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTTCGAATA	2400
AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT	GAATACTACT	2460
GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT	TGTTTAAACC	AGAAAACATT	2520
ACTTTTGAAG	ATGGGCACAGA	TCTTTTCATT	GCTATTTCAGG	CTGTTGATAA	GGTCGATCTG	2580
AAATCAGAAA	TATCCAACAT	TGCACGAGTA	TCTTTGTTTA	TTCTCTCCCA	GACTCCGCCA	2640
GAGACACCTA	GTCCTGATGA	AACGCTCTGCT	CCTTGTCTTA	ATATTTCATAT	CAACAGCACC	2700
ATTCTTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	TAGGAGAACT	GCAGCTGTCA	2760
ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAATAAA	TCATTTCATCC	TTA	2813

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1683 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT	CAAGAACTAG	60
AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC	TTCAGATCAA	GTTTCAGAACA	120
ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	180
CCATCCAGCT	TGAGAGTAAG	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	240
TGATCGTGGA	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC	300
CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGT	GACAGAAGCA	AGGTGGCTTT	GTAAGTGGACA	360
AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC	TAAGGTTGGC	ACTTGGAAAT	420
ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	480
CTACCCTGCC	TCCAATTACA	GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	540
GCCCTCTGGT	AGTTTATGCA	AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG	600
TCACAGCCCT	GATTGAATCA	GTGAATGGAA	AAACAGTTAC	CTTGGAACTA	CTGGATAATG	660
GAGCAGGTGC	TGATGCTACT	AAGGATGACG	GTGTCTACTG	AAGGTATTTT	ACAACCTATG	720
ACACGAATGG	TAGATACAGT	GTAAAAGTGC	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	780
GGAGAGTGAT	ACCCCGAGCAG	AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	840
AAATACAATG	GAATCCACCA	AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG	900
TGTGTTTCAG	CAGAACATCC	TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC	CCAAATGCTC	960
CCATACCTGA	TCTCTTCCCA	CCTGGCCAAA	TCACCGACCT	GAAGGCGGAA	ATTAATCGGG	1020
GCAGTCTCAT	TAATCTGACT	TGGACAGCTC	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	1080
ACAAGTATAT	CATTTCGAATA	AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	1140
CTCTTCAAGT	GAATACTACT	GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT	1200
TGTTTAAACC	AGAAAACATT	ACTTTTGAAG	ATGGCACAGA	TCTTTTCATT	GCTATTTCAGG	1260
CTTTTGATAA	GGTCGATCTG	AAATCAGAAA	TATCCAACAT	TGCACGAGTA	TCTTTGTTTA	1320
TTCTCTCCCA	GACTCCGCCA	GAGACACCTA	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	1380

ATATTCATAT	CAACAGCACC	ATTCCTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	1440
TAGGAGAACT	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA	1500
TCATTCATCC	TTTTTTTTTGA	TTATAAAATT	TTCTAAAATG	TATTTTAGAC	TTCTGTAGG	1560
GGGCGATATA	CTAAATGTAT	ATAGTACATT	TATACTAAAT	GTATTCCTGT	AGGGGGCGAT	1620
ATACTAAATG	TATTTTAGAC	TTCTGTAGG	GGGCGATAAA	ATAAAATGCT	AAACAAGTGG	1680
GTA						1683

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2983 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAAATCACAG	GGAGATGTAC	AGCAATGGGG	CCATTTAAGA	GTTCTGTGTT	CATCTTGATT	60
CTTCACCTTC	TAGAAGGGGC	CCTGAGTAAT	TCACTCATTC	AGCTGAACAA	CAATGGCTAT	120
GAAGGCATTG	TCGTTGCAAT	CGACCCCAAT	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	180
ATAAAGGACA	TGGTGACCCA	GGCATCTCTG	TATCTGTTTG	AAGCTACAGG	AAAGCGATTT	240
TATTTCAAAA	ATGTTGCCAT	TTTGATTCCCT	GAAACATGGA	AGACAAAGGC	TGACTATGTG	300
AGACCAAAAC	TTGAGACCTA	CAAAAATGCT	GATGTTCTGG	TTGCTGAGTC	TACTCCTCCA	360
GGTAATGATG	AACCCCTACAC	TGAGCAGATG	GGCAACTGTG	GAGAGAAGGG	TGAAAGGATC	420
CACCTCACTC	CTGATTTTCAT	TGCAGGAAAA	AAGTTAGCTG	AATATGGACC	ACAAGGTAGG	480
GCATTTGTCC	ATGAGTGGGC	TCATCTACGA	TGGGGAGTAT	TTGACGAGTA	CAATAATGAT	540
GAGAAATTCT	ACTTATCCAA	TGGAAGAATA	CAAGCAGTAA	GATGTTTCAGC	AGGTATTACT	600
GGTACAAATG	TAGTAAAGAA	GTGTCAGGGA	GGCAGCTGTT	ACACCAAAAG	ATGCACATTC	660
AATAAAGTAA	CAGGACTCTA	TGAAAAAGGA	TGTGAGTTTG	TTCTCCAATC	CCGCCAGACG	720
GAGAAGGCCT	CTATAATGTT	TGCACAACAT	GTTGATTCTA	TAGTTGAATT	CTGTACAGAA	780
CAAAACCACA	ACAAAGAAGC	TCCAAACAAG	CAAAATCAAA	AATGCAATCT	CCGAAGCACA	840
TGGGAAGTGA	TCCGTGATTG	TGAGGACTTT	AAGAAAACCA	CTCCTATGAC	AACACAGCCA	900
CCAAATCCCA	CCTTCTCATT	GCTGCAGATT	GGACAAAGAA	TTGTGTGTTT	AGTCCTTGAC	960
AAATCTGGAA	GCATGGCGAC	TGGTAACCGC	CTCAATCGAC	TGAATCAAGC	AGGCCAGCTT	1020
TTCTGTCTGC	AGACAGTTGA	GCTGGGGTCC	TGGGTTGGGA	TGGTGACATT	TGACAGTGCT	1080
GCCCATGTAC	AAAGTGAACT	CATACAGATA	AACAGTGGCA	GTGACAGGGA	CACACTCGCC	1140
AAAAGATTAC	CTGCAGCAGC	TTCAGGAGGG	ACGTCCATCT	GCAGCGGGCT	TCGATCGGCA	1200
TTTACTGTGA	TTAGGAAGAA	ATATCCAAT	GATGGATCTG	AAATTGTGCT	GCTGACGGAT	1260
GGGGAAGACA	ACACTATAAG	TGGGTGCTTT	AACGAGGTCA	AACAAAGTGG	TGCCATCATC	1320
CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT	CAAGAAGTAG	AGGAGCTGTC	CAAAATGACA	1380
GGAGGTTTAC	AGACATATGC	TTCAGATCAA	GTTTCAGAAC	ATGGCCTCAT	TGATGCTTTT	1440
GGGGCCCTTT	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG	1500
GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA	CAGCACCGTG	1560
GGAAAGGACA	CTTTGTTTTCT	TATCACCTGG	ACAACGCAGC	CTCCCCAAAT	CCTTCTCTGG	1620
GATCCCAGTG	GACAGAAGCA	AGGTGGCTTT	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	1680
TCCCAAATCC	CAGGCATTGC	TAAGGTTGGC	ACTTGGAAAT	ACAGTCTGCA	AGCAAGCTCA	1740
CAAACCTTGA	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA	1800
GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	GCCCTCTGGT	AGTTTATGCA	1860
AATATTTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG	TCACAGCCCT	GATTGAATCA	1920
TGTAATGGAA	AAACAGTTAC	CTTGGAACCTA	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	1980
AAGGATGACG	GTGTCTACTC	AAGGTATTTT	ACAACCTTATG	ACACGAATGG	TAGATACAGT	2040
GTAAAAGTGC	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCAGCAG	2100
AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG	GAATCCACCA	2160
AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG	TGTGTTTCAG	CAGAACATCC	2220
TCGGGAGGCT	CATTGTGTC	TTCTGATGTC	CCAAATGCTC	CCATACCTGA	TCTCTTCCCA	2280
CCTGGCCAAA	TCACCGACCT	GAAGGCGGAA	ATTACGCGGG	GCAGTCTCAT	TAATCTGACT	2340
TGGACAGCTC	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTCTGAATA	2400
AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT	GAATACTACT	2460
GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAATCTTTT	TGTTTAAACC	AGAAAAACATT	2520
ACTTTTGAAA	ATGGCACAGA	TCTTTTCATT	GCTATTTCAGG	CTGTTGATAA	GGTCGATCTG	2580
AAATCAGAAA	TATCCAACAT	TGCACGAGTA	TCTTTGTTTA	TTCTCCACA	GACTCCGCCA	2640
GAGACACCTA	GTCCTGATGA	AACGTCTGCT	CCTTGTCCTA	ATATTCATAT	CAACAGCACC	2700
ATTCCTGGCA	TTACATTTT	AAAAATTATG	TGGAAGTGA	TAGGAGAAGT	GCAGCTGTCA	2760
ATAGCTTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAATAAA	TCATTCATCC	TTTTTTTTTGA	2820
TTATAAAATT	TTCTAAAATG	TATTTTAGAC	TTCTGTAGG	GGGCGATATA	CTAAATGTAT	2880

06049696 "032798

ATAGTACATT TATACTAAAT GTATTCCTGT AGGGGGCGAT ATACTAAATG TATTTTAGAC 2940
TTCCTGTAGG GGGCGATAAA ATAAAATGCT AAACAACCTGG GTA 2983

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGCTCGGAAT TCCGAGCTTG GATCCTCTAG AGCGGCCGCC GACTAGTGAG CTCGTCGACC 60
CGGGAATT 68

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATTAATTCC CGGGTCGACG AGCTCACTAG TCGGCGGCCG CTCTAGAGGA TCCAAGCTCG 60
GAATTCCG 68

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCGGATAAC AATTTACAC AGGA 24

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGTAAACGA CGGCCAGT 18

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

09049656-035964060

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GAAGCAAGGT GGCTTTGTAG

20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGCTCACAA ACCTTGACCC

20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TACAGTGTA AAGTGCGGGC

20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCTGGGGATG ATTATGACCA

20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACGTCTGCTC CTTGTCCTAA

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

004936-0373

GCAGACGTTT CATCAGGACT

20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGGGAAGAGA TCAGGTATGG

20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGAGCCCGC ACTTTTACAC

20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACAGTCAGGG TCAAGGTTTG

20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CTGGAGGGTT AATCCCTTAC

20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

0504936-95954050

TCCTATGACA ACACAGCCAC

20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GATGTTCTGG TTGCTGAGTC

20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGCTGTGTTG TCATAGGAGT G

21

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGTTCATCAT TACCTGGAGG

20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TCCACCTCAC TCCTGATTTC ATTGC

25

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGATTCAGTC GATTGAGGCG GTTAC

25

00495964060

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 914 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met	Gly	Pro	Phe	Lys	Ser	Ser	Val	Phe	Ile	Leu	Ile	Leu	His	Leu	Leu
1				5					10					15	
Glu	Gly	Ala	Leu	Ser	Asn	Ser	Leu	Ile	Gln	Leu	Asn	Asn	Asn	Gly	Tyr
			20					25					30		
Glu	Gly	Ile	Val	Val	Ala	Ile	Asp	Pro	Asn	Val	Pro	Glu	Asp	Glu	Thr
		35					40					45			
Leu	Ile	Gln	Gln	Ile	Lys	Asp	Met	Val	Thr	Gln	Ala	Ser	Leu	Tyr	Leu
		50				55					60				
Phe	Glu	Ala	Thr	Gly	Lys	Arg	Phe	Tyr	Phe	Lys	Asn	Val	Ala	Ile	Leu
65					70					75				80	
Ile	Pro	Glu	Thr	Trp	Lys	Thr	Lys	Ala	Asp	Tyr	Val	Arg	Pro	Lys	Leu
				85					90					95	
Glu	Thr	Tyr	Lys	Asn	Ala	Asp	Val	Leu	Val	Ala	Glu	Ser	Thr	Pro	Pro
			100				105						110		
Gly	Asn	Asp	Glu	Pro	Tyr	Thr	Glu	Gln	Met	Gly	Asn	Cys	Gly	Glu	Lys
		115					120					125			
Gly	Glu	Arg	Ile	His	Leu	Thr	Pro	Asp	Phe	Ile	Ala	Gly	Lys	Lys	Leu
		130				135					140				
Ala	Glu	Tyr	Gly	Pro	Gln	Gly	Arg	Ala	Phe	Val	His	Glu	Trp	Ala	His
145					150					155					160
Leu	Arg	Trp	Gly	Val	Phe	Asp	Glu	Tyr	Asn	Asn	Asp	Glu	Lys	Phe	Tyr
				165					170					175	
Leu	Ser	Asn	Gly	Arg	Ile	Gln	Ala	Val	Arg	Cys	Ser	Ala	Gly	Ile	Thr
			180					185					190		
Gly	Thr	Asn	Val	Val	Lys	Lys	Cys	Gln	Gly	Gly	Ser	Cys	Tyr	Thr	Lys
		195					200					205			
Arg	Cys	Thr	Phe	Asn	Lys	Val	Thr	Gly	Leu	Tyr	Glu	Lys	Gly	Cys	Glu
		210				215					220				
Phe	Val	Leu	Gln	Ser	Arg	Gln	Thr	Glu	Lys	Ala	Ser	Ile	Met	Phe	Ala
225					230					235					240
Gln	His	Val	Asp	Ser	Ile	Val	Glu	Phe	Cys	Thr	Glu	Gln	Asn	His	Asn
				245					250					255	
Lys	Glu	Ala	Pro	Asn	Lys	Gln	Asn	Gln	Lys	Cys	Asn	Leu	Arg	Ser	Thr
			260					265					270		
Trp	Glu	Val	Ile	Arg	Asp	Ser	Glu	Asp	Phe	Lys	Lys	Thr	Thr	Pro	Met
		275					280					285			
Thr	Thr	Gln	Pro	Pro	Asn	Pro	Thr	Phe	Ser	Leu	Leu	Gln	Ile	Gly	Gln
		290				295					300				
Arg	Ile	Val	Cys	Leu	Val	Leu	Asp	Lys	Ser	Gly	Ser	Met	Ala	Thr	Gly
305					310					315					320
Asn	Arg	Leu	Asn	Arg	Leu	Asn	Gln	Ala	Gly	Gln	Leu	Phe	Leu	Leu	Gln
				325					330					335	
Thr	Val	Glu	Leu	Gly	Ser	Trp	Val	Gly	Met	Val	Thr	Phe	Asp	Ser	Ala
			340					345				350			
Ala	His	Val	Gln	Ser	Glu	Leu	Ile	Gln	Ile	Asn	Ser	Gly	Ser	Asp	Arg
		355				360						365			
Asp	Thr	Leu	Ala	Lys	Arg	Leu	Pro	Ala	Ala	Ala	Ser	Gly	Gly	Thr	Ser
		370				375					380				
Ile	Cys	Ser	Gly	Leu	Arg	Ser	Ala	Phe	Thr	Val	Ile	Arg	Lys	Lys	Tyr
385					390					395					400
Pro	Thr	Asp	Gly	Ser	Glu	Ile	Val	Leu	Leu	Thr	Asp	Gly	Glu	Asp	Asn
				405					410					415	

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Thr	Ile	Ser	Gly	Cys	Phe	Asn	Glu	Val	Lys	Gln	Ser	Gly	Ala	Ile	Ile		
			420					425					430				
His	Thr	Val	Ala	Leu	Gly	Pro	Ser	Ala	Ala	Gln	Glu	Leu	Glu	Glu	Leu		
		435					440					445					
Ser	Lys	Met	Thr	Gly	Gly	Leu	Gln	Thr	Tyr	Ala	Ser	Asp	Gln	Val	Gln		
	450					455					460						
Asn	Asn	Gly	Leu	Ile	Asp	Ala	Phe	Gly	Ala	Leu	Ser	Ser	Gly	Asn	Gly		
465					470					475					480		
Ala	Val	Ser	Gln	Arg	Ser	Ile	Gln	Leu	Glu	Ser	Lys	Gly	Leu	Thr	Leu		
			485						490					495			
Gln	Asn	Ser	Gln	Trp	Met	Asn	Gly	Thr	Val	Ile	Val	Asp	Ser	Thr	Val		
			500					505					510				
Gly	Lys	Asp	Thr	Leu	Phe	Leu	Ile	Thr	Trp	Thr	Thr	Gln	Pro	Pro	Gln		
		515					520					525					
Ile	Leu	Leu	Trp	Asp	Pro	Ser	Gly	Gln	Lys	Gln	Gly	Gly	Phe	Val	Val		
	530					535					540						
Asp	Lys	Asn	Thr	Lys	Met	Ala	Tyr	Leu	Gln	Ile	Pro	Gly	Ile	Ala	Lys		
545					550					555					560		
Val	Gly	Thr	Trp	Lys	Tyr	Ser	Leu	Gln	Ala	Ser	Ser	Gln	Thr	Leu	Thr		
			565						570					575			
Leu	Thr	Val	Thr	Ser	Arg	Ala	Ser	Asn	Ala	Thr	Leu	Pro	Pro	Ile	Thr		
			580					585						590			
Val	Thr	Ser	Lys	Thr	Asn	Lys	Asp	Thr	Ser	Lys	Phe	Pro	Ser	Pro	Leu		
		595					600					605					
Val	Val	Tyr	Ala	Asn	Ile	Arg	Gln	Gly	Ala	Ser	Pro	Ile	Leu	Arg	Ala		
	610				615						620						
Ser	Val	Thr	Ala	Leu	Ile	Glu	Ser	Val	Asn	Gly	Lys	Thr	Val	Thr	Leu		
625					630					635					640		
Glu	Leu	Leu	Asp	Asn	Gly	Ala	Gly	Ala	Asp	Ala	Thr	Lys	Asp	Asp	Gly		
			645						650					655			
Val	Tyr	Ser	Arg	Tyr	Phe	Thr	Thr	Tyr	Asp	Thr	Asn	Gly	Arg	Tyr	Ser		
			660					665					670				
Val	Lys	Val	Arg	Ala	Leu	Gly	Gly	Val	Asn	Ala	Ala	Arg	Arg	Arg	Val		
		675					680					685					
Ile	Pro	Gln	Gln	Ser	Gly	Ala	Leu	Tyr	Ile	Pro	Gly	Trp	Ile	Glu	Asn		
	690				695						700						
Asp	Glu	Ile	Gln	Trp	Asn	Pro	Pro	Arg	Pro	Glu	Ile	Asn	Lys	Asp	Asp		
705					710					715				720			
Val	Gln	His	Lys	Gln	Val	Cys	Phe	Ser	Arg	Thr	Ser	Ser	Gly	Gly	Ser		
			725						730					735			
Phe	Val	Ala	Ser	Asp	Val	Pro	Asn	Ala	Pro	Ile	Pro	Asp	Leu	Phe	Pro		
			740					745					750				
Pro	Gly	Gln	Ile	Thr	Asp	Leu	Lys	Ala	Glu	Ile	His	Gly	Gly	Ser	Leu		
		755					760					765					
Ile	Asn	Leu	Thr	Trp	Thr	Ala	Pro	Gly	Asp	Asp	Tyr	Asp	His	Gly	Thr		
	770					775					780						
Ala	His	Lys	Tyr	Ile	Ile	Arg	Ile	Ser	Thr	Ser	Ile	Leu	Asp	Leu	Arg		
785					790					795					800		
Asp	Lys	Phe	Asn	Glu	Ser	Leu	Gln	Val	Asn	Thr	Thr	Ala	Leu	Ile	Pro		
			805						810					815			
Lys	Glu	Ala	Asn	Ser	Glu	Glu	Val	Phe	Leu	Phe	Lys	Pro	Glu	Asn	Ile		
			820					825					830				
Thr	Phe	Glu	Asn	Gly	Thr	Asp	Leu	Phe	Ile	Ala	Ile	Gln	Ala	Val	Asp		
		835					840					845					
Lys	Val	Asp	Leu	Lys	Ser	Glu	Ile	Ser	Asn	Ile	Ala	Arg	Val	Ser	Leu		
		850				855					860						
Phe	Ile	Pro	Pro	Gln	Thr	Pro	Pro	Glu	Thr	Pro	Ser	Pro	Asp	Glu	Thr		
865					870					875					880		
Ser	Ala	Pro	Cys	Pro	Asn	Ile	His	Ile	Asn	Ser	Thr	Ile	Pro	Gly	Ile		
			885						890					895			
His	Ile	Leu	Lys	Ile	Met	Trp	Lys	Trp	Ile	Gly	Glu	Leu	Gln	Leu	Ser		
			900					905					910				
Ile	Ala																

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Tyr	Asn	Asn	Asp	Glu	Lys	Phe	Tyr	Leu	Ser	Asn	Gly	Arg	Ile	Gln	Ala
1				5					10					15	
Val	Arg	Cys													

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Tyr	Thr	Lys	Arg	Cys	Thr	Phe	Asn	Lys	Val	Thr	Gly	Leu	Tyr	Glu	Lys
1				5					10					15	
Gly	Cys	Glu	Phe	Val	Leu	Gln	Ser								
				20											

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asn	Ser	Gly	Ser	Asp	Arg	Asp	Thr	Leu	Ala	Lys	Arg	Leu	Pro	Ala	Ala
1				5					10					15	
Ala	Ser	Gly	Gly												
				20											

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Thr	Trp	Thr	Thr	Gln	Pro	Pro	Gln	Ile	Leu	Leu	Trp	Asp	Pro	Ser	Gly
1				5					10					15	
Gln	Lys	Gln	Gly	Gly	Phe	Val	Val	Asp	Lys	Asn	Thr	Lys			
				20				25							

004969 95964060

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Ile	Thr	Val	Thr	Ser	Lys	Thr	Asn	Lys	Asp	Thr	Ser	Lys	Phe	Pro	Ser
1				5					10					15	
Pro	Leu	Val	Val	Tyr	Ala	Asn	Ile	Arg	Gln	Gly	Ala	Ser			
			20					25							

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Asp	Asn	Gly	Ala	Gly	Ala	Asp	Ala	Thr	Lys	Asp	Asp	Gly	Val	Tyr	Ser
1				5					10					15	
Arg	Tyr	Phe	Thr	Thr	Tyr	Asp	Thr	Asn	Gly	Arg	Tyr	Ser	Val	Lys	
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Trp	Ile	Glu	Asn	Asp	Glu	Ile	Gln	Trp	Asn	Pro	Pro	Arg	Pro	Glu	Ile
1				5					10					15	
Asn	Lys	Asp	Asp	Val	Gln	His	Lys	Gln	Val	Cys	Phe	Ser	Arg	Thr	Ser
			20					25					30		
Ser	Gly	Gly													
			35												

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

00464060" 96964060

Glu Ile Asn Lys Asp Asp Val Gln His Lys Gln Val Cys Phe Ser Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Met His Thr Glu His
1 5 10 15
His His His His His
20

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selected from the group consisting of SEQUENCE ID NOS 41-49, and fragments thereof.

17. A gene, or a fragment thereof, which codes for a CS194 protein
5 comprising an amino acid sequence that has at least 60% identity with SEQUENCE ID NO 41.

18. A gene, or a fragment thereof, comprising DNA having at least 50%
identity with SEQUENCE ID NO 18, SEQUENCE ID NO 19, or SEQUENCE ID NO
10 20.

352220" 96964060

REAGENTS AND METHODS USEFUL FOR DETECTING
DISEASES OF THE GASTROINTESTINAL TRACT

5

Abstract of the Disclosure

10 A set of contiguous and partially overlapping cDNA sequences and polypeptides
encoded thereby, designated as CS194 and transcribed from GI tract tissue, is
described. These sequences are useful for the detecting, diagnosing, staging,
monitoring, prognosticating, preventing or treating, or determining the predisposition
of an individual to diseases and conditions of the GI tract, such as GI tract cancer. Also
provided are antibodies which specifically bind to CS194-encoded polypeptide or
protein, and agonists or inhibitors which prevent action of the tissue-specific CS194
15 polypeptide, which molecules are useful for the therapeutic treatment of GI tract
diseases, tumors or metastases.

88/2220-0364050

Figure 1-A

>1737775	GAAATCACAG	GGAGATGTAC	AGCAATGGGG	CCATTTAAGA	GTTCTGTGTT
>1737775IH	GAAATCACAG	GGAGATGTAC	AGCAATGGGG	CCATTTAAGA	GTTCTGTGTT
Consensus	GAAATCACAG	GGAGATGTAC	AGCAATGGGG	CCATTTAAGA	GTTCTGTGTT
>1737775	CATCTTGATT	CTTCACCTTC	TAGAAGGGGC	CCTGAGTAAT	TCACTCATT
>1737775IH	CATCTTGATT	CTTCACCTTC	TAGAAGGGGC	CCTGAGTAAT	TCACTCATT
Consensus	CATCTTGATT	CTTCACCTTC	TAGAAGGGGC	CCTGAGTAAT	TCACTCATT
>1737775	AGCTGAACAA	CAATGGCTAT	GAAGGCATTG	TCGTTGCAAT	CGACCCCAAT
>1737775IH	AGCTGAACAA	CAATGGCTAT	GAAGGCATTG	TCGTTGCAAT	CGACCCCAAT
>776455				GTTGCAAT	CGACCCCAAT
Consensus	AGCTGAACAA	CAATGGCTAT	GAAGGCATTG	TCGTTGCAAT	CGACCCCAAT
>1737775	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	ATAAAGGACA	TGGTGACCCA
>1737775IH	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	ATAAAGGACA	TGGTGACCCA
>776455	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	ATAAAGGACA	TGGTGACCCA
Consensus	GTGCCAGAAG	ATGAAACACT	CATTCAACAA	ATAAAGGACA	TGGTGACCCA
>1737775	GGCATCTCTG	TATCTGTTTG	AAG		
>1737775IH	GGCATCTCTG	TATCTGTTTG	AAGCTACAGG	AAAGCGATTT	TATTTCAAAA
>776455	GGCATCTCTG	TATCTGTTTG	AAGCTACAGG	AAAGCGATTT	TATTTCAAAA
Consensus	GGCATCTCTG	TATCTGTTTG	AAGCTACAGG	AAAGCGATTT	TATTTCAAAA
>1737775IH	ATGTTGCCAT	TTTGATTTCCT	GAAACATGGA	AGACAAAGGC	TGACTATGTG
>776455	ATGTTGCCAT	TTTGATTTCCT	GAAACATGGA	AGACAAAGGN	TGACTATGTG
Consensus	ATGTTGCCAT	TTTGATTTCCT	GAAACATGGA	AGACAAAGGC	TGACTATGTG
>1737775IH	AGACCAAAAC	TTGAGACCTA	CAAAAATGCT	GATGTTCTGG	TTGCTGAGTC
>776455	AGACCAAAAC	TTGAGACCTA	CAAAAATGCT	GATGTTCTGG	TTGCTGAGTC
>3234527			AAAATGCT	GATGTTCTGG	TTGCTGAGTC
Consensus	AGACCAAAAC	TTGAGACCTA	CAAAAATGCT	GATGTTCTGG	TTGCTGAGTC
>1737775IH	TACTCCTCCA	GGTAATGATG	AACCCTACAC	TGAGCAGATG	GGCAACTGTG
>776455	TANTCCTCCA	GGNAATGATG	AACCCTACAC	TGNGCAGATG	GGCAACTGTG
>3234527	TACTCCTCCA	GGTAATGATG	AACCCTACAC	TGAGCAGATG	GGCAACTGTG
Consensus	TACTCCTCCA	GGTAATGATG	AACCCTACAC	TGAGCAGATG	GGCAACTGTG
>1737775IH	GAGAGAAGGG	TGAAAGGATC	CACCTCACTC	CTGATTTTCAT	TGCAGGAAAA
>776455	GCGAG				
>3234527	GAGAGAAGGG	TGAAAGGATC	CACCTCACTC	CTGATTTTCAT	TGCAGGAAAA
Consensus	GAGAGAAGGG	TGAAAGGATC	CACCTCACTC	CTGATTTTCAT	TGCAGGAAAA
>1737775IH	AAGTTAGCTG	AATATGGACC	ACAAGGTAGG	GCATTTGTCC	ATGAGTGGGC
>3234527	AAGTTAGCTG	AATATGGACC	ACAAGGTAGG	GCATTTGTCC	ATGAGTGGGC
Consensus	AAGTTAGCTG	AATATGGACC	ACAAGGTAGG	GCATTTGTCC	ATGAGTGGGC
>1737775IH	TCATCTACGA	TGGGGAGTAT	TTGACGAGTA	CAATAATGAT	GAGAAATTCT
>3234527	TCATCTACGA	TGGGGAGTAT	TTGACGAGTA	CAATAATGAT	GAGAAATTCT
Consensus	TCATCTACGA	TGGGGAGTAT	TTGACGAGTA	CAATAATGAT	GAGAAATTCT

Figure 1-B

>1737775IH	ACTTATCCAA	TGGAAGAATA	CAAGCAGTAA	GATGTTTCAGC	AGGTATTACT
>3234527	ACTTATCCAA	TG			
Consensus	ACTTATCCAA	TGGAAGAATA	CAAGCAGTAA	GATGTTTCAGC	AGGTATTACT
>1737775IH	GGTACAAATG	TAGTAAAGAA	GTGTCAGGGA	GGCAGCTGTT	ACACCAAAAG
>1628724					CAAAAG
Consensus	GGTACAAATG	TAGTAAAGAA	GTGTCAGGGA	GGCAGCTGTT	ACACCAAAAG
>1737775IH	ATGCACATTC	AATAAAGTAA	CAGGACTCTA	TGAAAAAGGA	TGTGAGTTTG
>1628724	ATGCACATTC	AATAAAGTAA	CAGGACTCTA	TGAAAAAGGA	TGTGAGTTTG
Consensus	ATGCACATTC	AATAAAGTAA	CAGGACTCTA	TGAAAAAGGA	TGTGAGTTTG
>1737775IH	TTCTCCAATC	CCGCCAGACG	GAGAAGGCTT	CTATAATGTT	TGCACAACAT
>1628724	TTCTCCAATC	CCGCCAGACG	GAGAAGGCTT	CTATAATGTT	TGCACAACAT
Consensus	TTCTCCAATC	CCGCCAGACG	GAGAAGGCTT	CTATAATGTT	TGCACAACAT
>1737775IH	GTTGATTCTA	TAGTTGAATT	CTGTACAGAA	CAAAACCACA	ACAAAGAAGC
>1628724	GTTGATTCTA	TAGTTGAATT	CTGTACAGAA	CAAAACCACA	ACAAAGAAGC
>1427224		CTA TAGTTGAATT	CTGTACAGAA	CAAAACCACA	ACAAAGAAGC
Consensus	GTTGATTCTA	TAGTTGAATT	CTGTACAGAA	CAAAACCACA	ACAAAGAAGC
>1737775IH	TCCAAACAAG	CAAAATCAAA	AATGCAATCT	CCGAAGCACA	TGGGAAGTGA
>1628724	TCCAAACAAG	CAAAATCAAA	AATGC		
>1427224	TCCAAACAAG	CAAAATCAAA	AATGCAATCT	CCGAAGCACA	TGGGAAGTGA
Consensus	TCCAAACAAG	CAAAATCAAA	AATGCAATCT	CCGAAGCACA	TGGGAAGTGA
>1737775IH	TCCGTGATTC	TGAGGACTTT	AAGAAAACCA	CTCCTATGAC	AACACAGCCA
>1427224	TCCGTGATTC	TGAGGACTTT	AAGAAAACCA	CTCCTATGAC	AACACAGCCA
Consensus	TCCGTGATTC	TGAGGACTTT	AAGAAAACCA	CTCCTATGAC	AACACAGCCA
>1737775IH	CCAAATCCCA	CCTTCTCATT	GCTGCAGATT	GGACAAAGAA	TTGTGTGTTT
>1427224	CCAAATCCCA	CCTTCTCATT	GCTGCAGATT	GGACAAAGAA	TTGTGTGTTT
>3235571				CAAAGAA	TTGTGTGTTT
Consensus	CCAAATCCCA	CCTTCTCATT	GCTGCAGATT	GGACAAAGAA	TTGTGTGTTT
>1737775IH	AGTCCTTGAC	AAATCTGGAA	GCATGGCGAC	TGGTAACCGC	CTCAATCGAC
>1427224	AGTCCTTGAC	AAATCTGGAA	GCATGGC		
>3235571	AGTCCTTGAC	AAATCTGGAA	GCATGGCGAC	TGGTAACCGC	CTCAATCGAC
Consensus	AGTCCTTGAC	AAATCTGGAA	GCATGGCGAC	TGGTAACCGC	CTCAATCGAC
>1737775IH	TGAATCAAGC	AGGCCAGCTT	TTCTTGCTGC	AGACAGTTGA	GCTGGGGTCC
>3235571	TGAATCAAGC	AGGCCAGCTT	TTCTTGCTGC	AGACAGTTGA	GCTGGGGTCC
Consensus	TGAATCAAGC	AGGCCAGCTT	TTCTTGCTGC	AGACAGTTGA	GCTGGGGTCC
>1737775IH	TGGGTGGGA	TGGTGACATT	TGACAGTGCT	GCCCATGTAC	AAAGTGAAC
>3235571	TGGGTGGGA	TGGTGACATT	TGACAGTGCT	GCCCATGTAC	AAAGTGAAC
Consensus	TGGGTGGGA	TGGTGACATT	TGACAGTGCT	GCCCATGTAC	AAAGTGAAC

Figure 1-C

>1737775IH	CATACAGATA	AACAGTGGCA	GTGACAGGGA	CACACTCGCC	AAAAGATTAC
>3235571	CATACAGATA	AACAGTGGCA	GTGACAGGGA	CACACTCGCC	AAAAGATTAC
Consensus	CATACAGATA	AACAGTGGCA	GTGACAGGGA	CACACTCGCC	AAAAGATTAC
>1737775IH	CTGCAGCAGC	TTCAGGAGGG	ACGTCCATCT	GCAGCGGGCT	TCGATCGGCA
>3235571	CTGCAGCAGC	TTCAGGAGGG	ACGTCCATCT	GCAGC	
Consensus	CTGCAGCAGC	TTCAGGAGGG	ACGTCCATCT	GCAGCGGGCT	TCGATCGGCA
>1737775IH	TTTACTGTGA	TTAGGAAGAA	ATATCCAAC	GATGGATCTG	AAATTGTGCT
>2680592		GAA	ATATCCAAC	GATGGATCTG	AAATTGTGCT
Consensus	TTTACTGTGA	TTAGGAAGAA	ATATCCAAC	GATGGATCTG	AAATTGTGCT
>1737775IH	GCTGACGGAT	GGGGAAGACA	ACACTATAAG	TGGGTGCTTT	AACGAGGTCA
>2680592	GCTGACGGAT	GGGGAAGACA	ACACTATAAG	TGGGTGCTTT	AACGAGGTCA
Consensus	GCTGACGGAT	GGGGAAGACA	ACACTATAAG	TGGGTGCTTT	AACGAGGTCA
>1737775IH	AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT
>2680592	AACAAAGTNG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT
>608819	AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT
>608819IH	AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT
Consensus	AACAAAGTGG	TGCCATCATC	CACACAGTCG	CTTTGGGGCC	CTCTGCAGCT
>1737775IH	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC
>2680592	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC
>608819	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC
>608819IH	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC
Consensus	CAAGAACTAG	AGGAGCTGTC	CAAAATGACA	GGAGGTTTAC	AGACATATGC
>1737775IH	TTCAGATCAA	GTTTCTGAG	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
>2680592	TTCAGATCAA	GTTTCTGAG	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
>608819	TTCAGATCAA	GTTTCTGAG	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
>608819IH	TTCAGATCAA	GTTTCTGAG	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
>1629002		GAACA	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
Consensus	TTCAGATCAA	GTTTCTGAG	ATGGCCTCAT	TGATGCTTTT	GGGGCCCTTT
>1737775IH	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG
>2680592	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG
>608819	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG
>608819IH	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG
>1629002	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG
Consensus	CATCAGGAAA	TGGAGCTGTC	TCTCAGCGCT	CCATCCAGCT	TGAGAGTAAG

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Figure 1-D

>1737775IH	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA
>2680592	GGATTA				
>608819	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA
>608819IH	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA
>1629002	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA
>1626435				GGCACAG	TGATCGTGGA
Consensus	GGATTAACCC	TCCAGAACAG	CCAGTGGATG	AATGGCACAG	TGATCGTGGA
>1737775IH	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC
>608819	CAG				
>608819IH	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC
>1629002	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC
>1626435	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC
Consensus	CAGCACCGTG	GGAAAGGACA	CTTTGTTTCT	TATCACCTGG	ACAACGCAGC
>1737775IH	CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGTG	GACAGAAGCA	AGGTGGCTTT
>608819IH	CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGTG	GACAGAAGCA	AGGTGGCTTT
>1629002	CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGTG	GAC	
>1626435	CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGTG	GACAGAAGCA	AGGTGGCTTT
>1582693					GGCTTT
Consensus	CTCCCCAAAT	CCTTCTCTGG	GATCCCAAGTG	GACAGAAGCA	AGGTGGCTTT
>1737775IH	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC
>608819IH	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC
>1626435	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC
>1582693	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC
Consensus	GTAGTGGACA	AAAACACCAA	AATGGCCTAC	CTCCAAATCC	CAGGCATTGC
>1737775IH	TAAGGTGGC	ACTTGGAAT	ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA
>608819IH	TAAGGTGGC	ACTTGGAAT	ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA
>1626435	TAAGGTGGC	ACTTGGAAT	ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA
>1582693	TAAGGTGGC	ACTTGGAAT	ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA
Consensus	TAAGGTGGC	ACTTGGAAT	ACAGTCTGCA	AGCAAGCTCA	CAAACCTTGA
>1737775IH	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA
>608819IH	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA
>1626435	CCCTGACTGT	CA			
>1582693	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA
Consensus	CCCTGACTGT	CACGTCCCGT	GCGTCCAATG	CTACCCTGCC	TCCAATTACA
>1737775IH	GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	GCCCTCTGGT
>608819IH	GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	GCCCTCTGGT
>1582693	GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATT	
>1281379			GACACCAGC	AAATTCCCCA	GCCCTCTGGT
Consensus	GTGACTTCCA	AAACGAACAA	GGACACCAGC	AAATTCCCCA	GCCCTCTGGT

Figure 1-E

>1737775IH	AGTTTATGCA	AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG
>608819IH	AGTTTATGCA	AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG
>1281379	AGTTTATGCA	AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG
Consensus	AGTTTATGCA	AATATTCGCC	AAGGAGCCTC	CCCAATTCTC	AGGGCCAGTG
>1737775IH	TCACAGCCCT	GATTGAATCA	GTGAATGGAA	AAACAGTTAC	CTTGGAACTA
>608819IH	TCACAGCCCT	GATTGAATCA	GTGAATGGAA	AAACAGTTAC	CTTGGAACTA
>1281379	TCACAGCCCT	GATTGAATCA	GTGAATGGAA	AAACAGTTAC	CTTGGAACTA
Consensus	TCACAGCCCT	GATTGAATCA	GTGAATGGAA	AAACAGTTAC	CTTGGAACTA
>1737775IH	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	AAGGATGACG	GTGTCTACTC
>608819IH	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	AAGGATGACG	GTGTCTACTC
>1281379	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	AAGGATGACG	GTGTCTACTC
Consensus	CTGGATAATG	GAGCAGGTGC	TGATGCTACT	AAGGATGACG	GTGTCTACTC
>1737775IH	AAGGTATTTT	ACAACCTTATG	ACACGAATGG	TAGATACAGT	GTAAGAGTGC
>608819IH	AAGGTATTTT	ACAACCTTATG	ACACGAATGG	TAGATACAGT	GTAAGAGTGC
>1281379	AAGGTATTTT	ACAACCTTATG	ACACGAATGG	TAGATACAGT	GTAAGAGTGC
>1628509					GTGC
Consensus	AAGGTATTTT	ACAACCTTATG	ACACGAATGG	TAGATACAGT	GTAAGAGTGC
>1737775IH	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCAGCAG
>608819IH	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCAGCAG
>1281379	GGGCTCT				
>1628509	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCAGCAG
Consensus	GGGCTCTGGG	AGGAGTTAAC	GCAGCCAGAC	GGAGAGTGAT	ACCCAGCAG
>1737775IH	AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG
>608819IH	AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG
>1628509	AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG
Consensus	AGTGGAGCAC	TGTACATACC	TGGCTGGATT	GAGAATGATG	AAATACAATG
>1737775IH	GAATCCACCA	AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG
>608819IH	GAATCCACCA	AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG
>1628509	GAATCCACCA	AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG
Consensus	GAATCCACCA	AGACCTGAAA	TTAATAAGGA	TGATGTTCAA	CACAAGCAAG
>1737775IH	TGTGTTTCAG	CAGAACATCC	TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC
>608819IH	TGTGTTTCAG	CAGAACATCC	TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC
>1628509	TGTGTTTCAG	CAGAACATCC	TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC
Consensus	TGTGTTTCAG	CAGAACATCC	TCGGGAGGCT	CATTTGTGGC	TTCTGATGTC
>1737775IH	CCAAATGCTC	CCATACCTGA	TCTCTTCCCA	CCTGGCCAAA	TCACCGACCT
>608819IH	CCAAATGCTC	CCATACCTGA	TCTCTTCCCA	CCTGGCCAAA	TCACCGACCT
>1628509	CCAAATGCTC	CCATACCTGA	TCT		
>2060739					ACCT
Consensus	CCAAATGCTC	CCATACCTGA	TCTCTTCCCA	CCTGGCCAAA	TCACCGACCT

Figure 1-F

>1737775IH	GAAGGCGGAA	ATTACGCGG	GCAGTCTCAT	TAATCTGACT	TGGACAGCTC
>608819IH	GAAGGCGGAA	ATTACGCGG	GCAGTCTCAT	TAATCTGACT	TGGACAGCTC
>2060739	GAAGGCGGAA	ATTACGCGG	GCAGTCTCAT	TAATCTGACT	TGGACAGCTC
Consensus	GAAGGCGGAA	ATTACGCGG	GCAGTCTCAT	TAATCTGACT	TGGACAGCTC
>1737775IH	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTCTGAATA
>608819IH	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTCTGAATA
>2060739	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTCTGAATA
Consensus	CTGGGGATGA	TTATGACCAT	GGAACAGCTC	ACAAGTATAT	CATTCTGAATA
>1737775IH	AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT
>608819IH	AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT
>2060739	AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT
Consensus	AGTACAAGTA	TTCTTGATCT	CAGAGACAAG	TTCAATGAAT	CTCTTCAAGT
>1737775IH	GAATACTACT	GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT
>608819IH	GAATACTACT	GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT
>2060739	GAATACTACT	GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT
Consensus	GAATACTACT	GCTCTCATCC	CAAAGGAAGC	CAACTCTGAG	GAAGTCTTTT
>1737775IH	TGTTTTAAACC	AGAAAACATT	ACTTTTGAAA	ATGGCACAGA	TCTTTTCATT
>608819IH	TGTTTTAAACC	AGAAAACATT	ACTTTTGAAA	ATGGCACAGA	TCTTTTCATT
>2060739	TGTTTTAAACC	AGAAAACATT	ACTTTTGAAA	ATGGCACAGA	TCTTT
>1624934				ACAGA	TCTTTTCATT
Consensus	TGTTTTAAACC	AGAAAACATT	ACTTTTGAAA	ATGGCACAGA	TCTTTTCATT
>1737775IH	GCTATTCAGG	CTGTTGATAA	GGTCGATCTG	AAATCAGAAA	TATCCAACAT
>608819IH	GCTATTCAGG	CTGTTGATAA	GGTCGATCTG	AAATCAGAAA	TATCCAACAT
>1624934	GCTATTCAGG	CTGTTGATAA	GGTCGATCTG	AAATCAGAAA	TATCCAACAT
Consensus	GCTATTCAGG	CTGTTGATAA	GGTCGATCTG	AAATCAGAAA	TATCCAACAT
>1737775IH	TGCACGAGTA	TCTTTGTTTA	TTCTCCACA	GACTCCGCCA	GAGACACCTA
>608819IH	TGCACGAGTA	TCTTTGTTTA	TTCTCCACA	GACTCCGCCA	GAGACACCTA
>1624934	TGCACGAGTA	TCTTTGTTTA	TTCTCCACA	GACTCCGCCA	GAGACACCTA
>1800229		GTTTA	TTCTCCACA	GACTCCGCCA	GAGACACCTA
Consensus	TGCACGAGTA	TCTTTGTTTA	TTCTCCACA	GACTCCGCCA	GAGACACCTA
>1737775IH	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	ATATTCATAT	CAACAGCACC
>608819IH	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	ATATTCATAT	CAACAGCACC
>1624934	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	ATATTCATA	
>1800229	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	ATATTCATAT	CAACAGCACC
Consensus	GTCCTGATGA	AACGTCTGCT	CCTTGTCTTA	ATATTCATAT	CAACAGCACC
>1737775IH	ATTCCCTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	TAGGAGAACT
>608819IH	ATTCCCTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	TAGGAGAACT
>1800229	ATTCCCTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	TAGGAGAACT
>990829		GGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA
Consensus	ATTCCCTGGCA	TTCACATTTT	AAAAATTATG	TGGAAGTGGA	TAGGAGAACT

Figure 1-G

>1737775IH	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA
>608819IH	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA
>1800229	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA
>990829	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA
Consensus	GCAGCTGTCA	ATAGCCTAGG	GCTGAATTTT	TGTCAGATAA	ATAAAAATAAA
>1737775IH	TCATTCATCC	TTA			
>608819IH	TCATTCATCC	TTTTTTTTTGA	TTATAAAATT	TTCTAAAATG	TATTTTAGAC
>1800229	TCATTCATCC	TTTTTTTTTGA	TTATAAAATT	TTCTAAAATG	TATTTTAGAC
>990829	TCATTCATCC	TT			
Consensus	TCATTCATCC	TTTTTTTTTGA	TTATAAAATT	TTCTAAAATG	TATTTTAGAC
>608819IH	TTCTGTAGG	GGGCGATATA	CTAAATGTAT	ATAGTACATT	TATACTAAAT
>1800229	TTCTGT				
Consensus	TTCTGTAGG	GGGCGATATA	CTAAATGTAT	ATAGTACATT	TATACTAAAT
>608819IH	GTATTCCTGT	AGGGGGCGAT	ATACTAAATG	TATTTTAGAC	TTCTGTAGG
Consensus	GTATTCCTGT	AGGGGGCGAT	ATACTAAATG	TATTTTAGAC	TTCTGTAGG
>608819IH	GGGCGATAAA	ATAAAAATGCT	AAACAACCTGG	GTA	
Consensus	GGGCGATAAA	ATAAAAATGCT	AAACAACCTGG	GTA	

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Figure 2

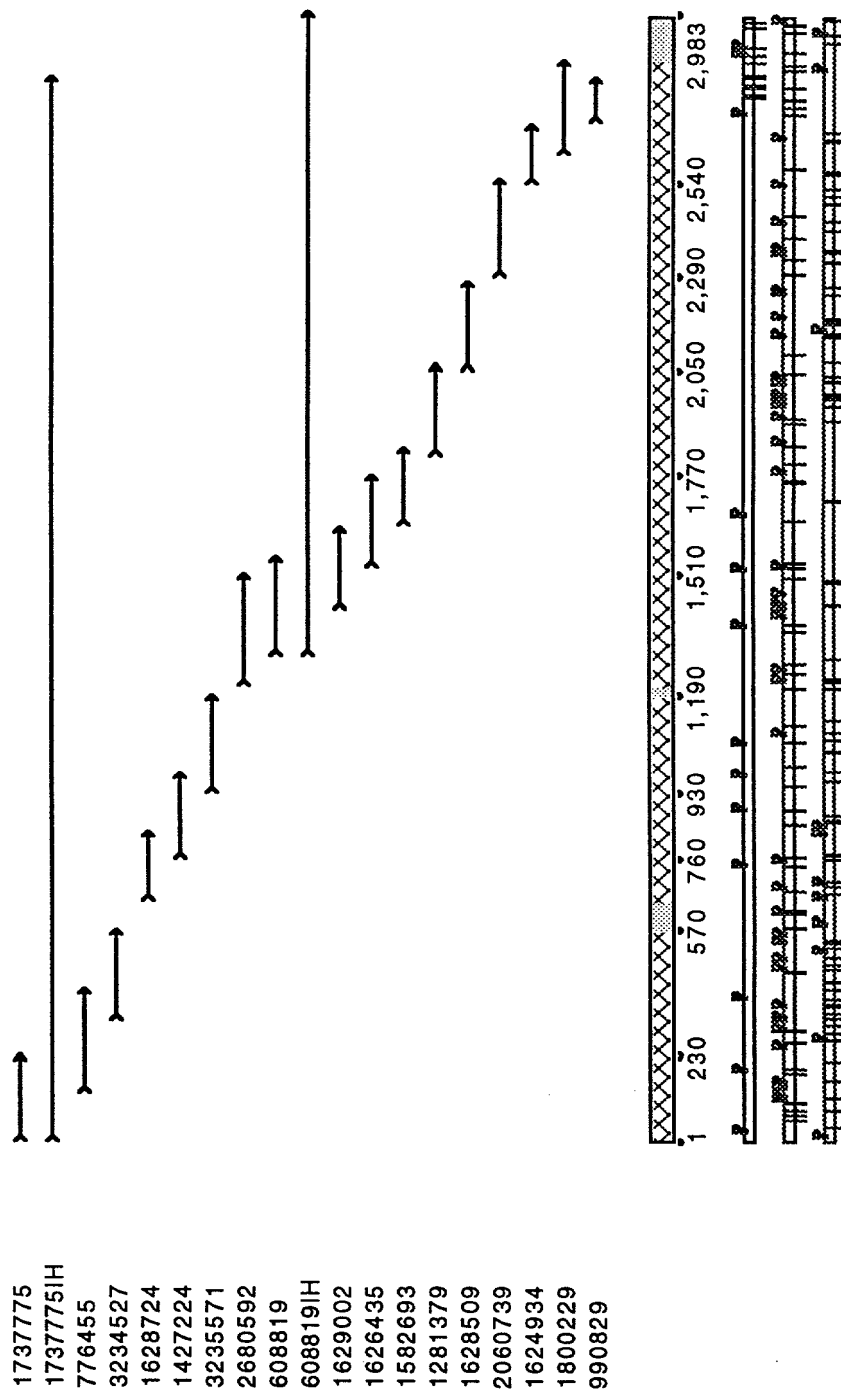
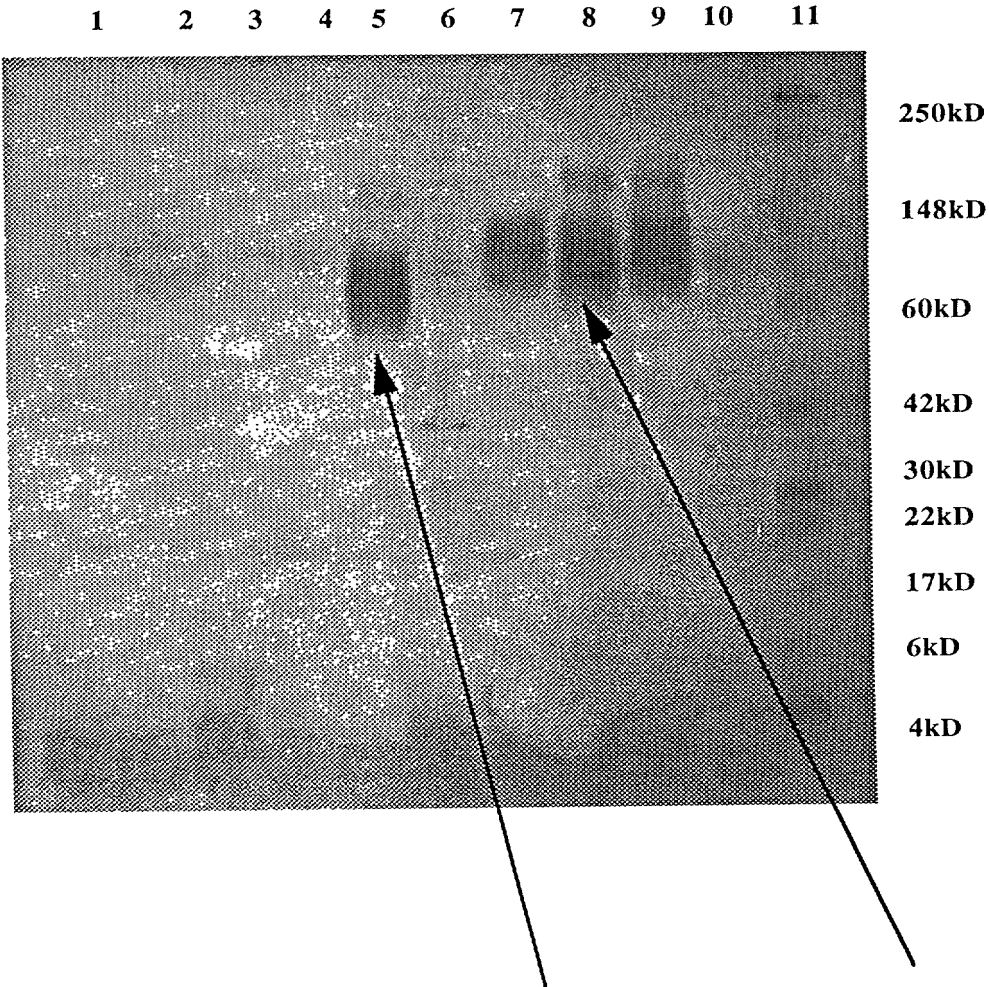


Figure 3



Lane	Tissue	Lane	Tissue
1	Prostate	7	Colon
2	Breast	8	Colon
3	Bladder	9	Colon
4	Lung	10	Blank
5	Colon cancer	11	Markers
6	Colon cancer		

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Filed: on even date herewith

**Title: REAGENTS AND METHODS
USEFUL FOR DETECTING DISEASES OF
THE GASTROINTESTINAL TRACT**

Case No.: 6067.US.P1

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DECLARATION AND POWER OF ATTORNEY FOR A UNITED STATES PATENT APPLICATION

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name. I believe that I am an original and first and joint inventor of the subject matter of the invention entitled REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE GASTROINTESTINAL TRACT, which is claimed and for which a patent is sought in the patent application attached hereto.

I hereby state that I have reviewed and understand the contents of the above-mentioned specification, including the claims.

I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Claim to benefit of foreign application(s):

I hereby claim foreign priority benefits under 35 U.S.C. §119 for the following foreign application(s)
for patent or inventor's certificate:

NONE

The following foreign applications for patent or inventor's certificate have a filing date earlier than the filing date of the application(s) identified above:

NONE

Claim to benefit of U.S. Application(s):

I hereby claim the benefit under 35 U.S.C. §120 of the following earlier-filed United States patent application(s):

U.S. Serial No. 08/829,754, filed March 31, 1997, pending.

Insofar as the subject matter of each of the claims of this/these application(s) is not disclosed in the prior U.S. Applications in the manner required by 35 U.S.C. § 112, first paragraph, I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. Which came into existence between the filing date(s) of the prior applications and the national or PCT filing date of this application.

I hereby appoint the following Attorneys and/or agents to prosecute this application and any continuation or divisional applications based hereon, and to transact all business in the Patent and Trademark Office connected therewith:

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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